Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/1870
DOI URL: http://dx.doi.org/10.21474/IJAR01/1870
ISSN NO. 2320-5407

RESEARCH ARTICLE

THE EFFECT OF WATER CONTENT ON DAMAGE DEPTH IN TISSUE SUBJECTED TO CO_2 LASER, AN EXPERMANTEL AND THEORITCAL STUDY

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Manuscript Info

Manuscript History

Received: 12 August 2016
Final Accepted: 22 September 2016
Published: October 2016

Key words:-
CW CO2 Laser, Water Content, Damage Depth, Tissue

Abstract

In this paper, the effect of subjecting CW CO_2 laser on three types of tissues, having different water content (muscle, brain and lung) have been studied and the damage depth in tissue subjected to CO_2 laser are found theoretically and experimentally. This paper, studied the thermal damage using two methods, thermal Dose (CEM_{43}) and Arrhenius' equation. From this work, it is found that: the damage depth that occurs due to apply CW CO_2 laser, is decreased with the increase of laser power, velocity, and water content in tissue. Arrhenius model is found to be more accurate than CEM_{43} based on the damage assessment. The results of this research provide useful information to the surgeon who aimed to decrease damage depth, which decrease healing time.

Introduction:-

The first CO_2 laser was developed by Patel(et al.)in 1964[1]. carbon dioxide CO_2 (10600 nm) laser is widely used as a scalpel in surgical operation and in other medical fields [2].For CW CO_2 laser the photons that absorb are the essential part of induced laser photons which cause a rapid increase in the temperature of tissue where many phenomenon may happen such as hyperthermia, denaturation of proteins, coagulation, and cells necrosis until the limit of water evaporation is reached [3]. This study aims to evaluate the damage depth produced by subjecting CO_2 laser on different types of tissue. Lasers, allow to obtaining an important advantages for the patient. In surgical field, it is possible to decrease the amount of local anesthesia and to obtain a faster postoperative cure; essentially, in the early stages[4].The damage depth of tissues can be studied using two methods Thermal Dose, and Arrhenius' equation [5].

Martin Kaplan (et al.), in 2015, have found that the ablation depth of CO_2 laser is controlled to a few tenths of a millimeter, which distinguish this wavelength as a safe soft tissue removal [6]. Andrew Jacono (et al.), in 2016, reported that the short-pulsed high energy and CW CO_2 lasers and other laser systems that limit skin heating have revolutionized laser skin resurfacing. These lasers are able to remove layers of damaged skin in an impressively accurate mode and leaving just a narrow region of thermal necrosis [7].

In this paper, the percentage of the water in different tissue, which is (muscle, brain and lung), had been determined by measuring the weight of each fresh tissue before and after drying in oven, it was found that brain tissue has water

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content more than muscle and lung tissue [8]. Each tissue sample was subjecting to different power CO_2 laser for 20 s, after that the tissue was prepared for examination under light microscope by fixation of the tissue on glass slide using (paraffin) embedding section method to determine damage depth using scaled optics [9]. This result was used in computer program to find the average thermal dose that was used in Thermal Dose (CEM_{43}) equation and Arrhenius' equation to determine the damage depth theoretically.

This work was found that damage depth decrease with increase each of power of CO_2 laser and water content. The depth in brain tissue is less than muscle and lung tissue the method of Arrhenius equation is more accurate than CEM_{43} equation.

Theory:-

Temperature distribution in tissue cut by CO_2 laser:-

Temperature distribution can be calculated by assuming a quasi -steady state ablation of tissue, assuming the thickness of tissue is very large compared with material removed then the one- dimensional equation can be written as [10]:

$$\frac{\partial^2 \mathrm{T}}{\partial \mathrm{y}^2} = \frac{1}{\alpha}\frac{\partial \mathrm{T}}{\partial \mathrm{t}} \quad (1)$$

Where:

T: temperature [°C],α: The thermal diffusivity [m/s^2], $= \frac{k}{\rho C}$ (2)

Where:

ρ: The density [kg/m^3], and for tissue it can be written as [10]:

$\rho = 1000/ (0.06w+0.938)$ (3)

w: is tissue water content ,*C*: specific heat in[J/kg.K], which can be written as:

$c=1000(2.5w+1.7)$ (4)

k: thermal conductivity in[W/m.k] which can be written as:

$k = \rho(0.45w+0.174)/1000$ (5)

The variables in eq. (1) can be changing into a moving coordinate system, following the transformation: $\varepsilon = y - v\tau$

v : Evaporation velocity, which is equal to the char ablation velocity, which is in the range (0.1 mm/s to 2 mm/s). Then the equation of one -dimensional can be converting into moving boundary system, as follow :

$$\frac{\partial^2 T}{\partial y^2} = \frac{\partial^2 T}{\partial \varepsilon^2} \quad (6)$$

And

$$\frac{\partial T}{\partial t} = -v\frac{\partial T}{\partial \varepsilon} \quad (7)$$

The result is:

$$\frac{\partial^2 T}{\partial \varepsilon^2} + v\frac{1}{\alpha}\frac{\partial T}{\partial \varepsilon} = 0 \quad (8)$$

The solution of equation (8) is :

$$T = C + C_1 Exp\left(-\frac{v\varepsilon}{\alpha}\right) \quad (9)$$

- At the surface of tissue, the evaporation of water is happened when the temperature is equal 100 °C, then:

$$T = T_m = 100^o C \quad at\varepsilon = 0 \quad (10)$$

- At far depth when $\varepsilon \to \infty$ then

$T = T_i$ (i.e. initial temperature which is equal to 25°C). Then, the solution can be rearranged as:

$$\frac{T-T_i}{T_m-T_i} = \exp\left(\frac{-v\varepsilon}{\alpha}\right) \quad (11)$$

The first depth of the tissue, which has 43°C, is calculated by using equation (11), when time is equal to zero, while, the thermal diffusivity is calculated by using equation (2)[10].

Methods of predication thermal damage depth:-

Cumulative Equivalent Minutes (Thermal Dose):-

Sapareto and Dewey define the CEM_{43} of thermal dose at 43°C which can describe the extent of thermal damage of tissue. It is a function of the temperature rise mand time, as described in equations [11](12):

$$CEM_{43}=\sum_{i=1}^{n} t\, R^{(43-Ti)} \quad (12)$$

Where:

$CEM_{43:}$ the cumulative number of equivalent minutes at 43 °C

t_i: the time interval [min]

T: the average temperature during time interval $t_{i..}$

R: related to the temperature dependence of the rate of cell death [11].

R=0.25 for T< 43 ℃ , R=0.5 for T>43 ℃

The equivalent minutes of critical thermal dose (CEM_{43}) of liver tissue is equal to 340 minutes. Therefore, such tissue is considered necroses, when the CEM_{43} exceeded 340 minutes[12].

Arrhenius' equation:-

Arrhenius formulation, in which the thermal damage in the tissue is described as a temperature dependent rate process. Arrhenius equation (13) calculates the accumulative damage in a tissue, exposed to a given temperature T for a specific time t:

$$\Omega\,(T;\,t) = A\int_{ti}^{tf} e^{-\frac{Ea}{R.T}}\, dt \quad (13)$$

Where

A: the frequency factor 1/s

$(t_f - t_i)$: the exposure time s

R: the universal gas constant J/mol.k

E_a : the energy activation barrier J/mol [13]

The Ω equal to 1 corresponds to a decrease in concentration of original molecules to 36% and it is always used for the threshold of irreversible tissue damage [14].

Experimental work:-

Measurement the percentage of water in tissues:-

The samples were prepared by cutting the sheep tissue samples into a rectangular shape piece with dimensions 1cm long, 0.5cm width and thick**,** as shown in Fig.1.

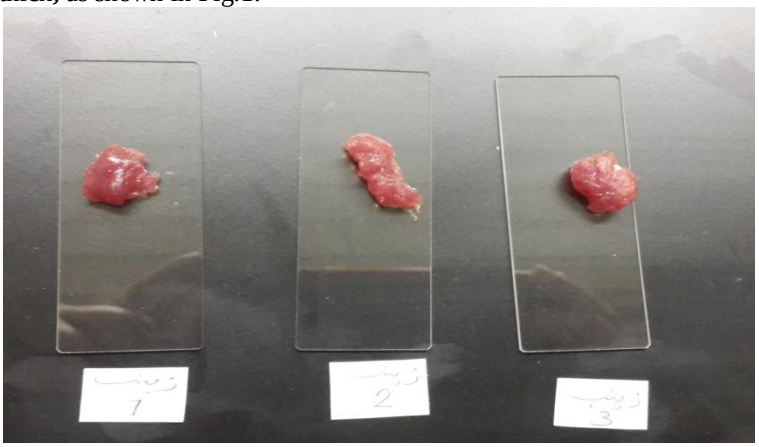

Figure 1:- Fresh sheep tissues with dimensions 1cm length, 0.5cm width and thick.

The cutting step followed by calculating the weight of each sample by using electrical sensitive balance (Mettler). Then the samples were placed in the oven in temperature 50°C for 10 min. and then the weight of each sample was calculated to determine the difference in weight that represents the weight of the water. This step was repeated

several times until the weight of tissue was fixed, then calculated the percentage of water in each tissue using equation (13) [8]:

$$\text{The percentage of water} = \frac{\text{Weight of fresh tissue} - \text{Weight of dray tissue}}{\text{Weight of fresh tissue}} \quad (13)$$

Experimental Measurement of damage depth:-

Set up of the work:-

1. CW CO_2 laser
2. Pieces of tissue from the sheep
3. Holder
4. Power meter.
5. Microscope
6. Scaled optics, as shown in Fig.2

Figure2:- the set up of work

The Experiment:-

In this work different sheep organs (age 6 months)was used(Brain ,Lung ,Muscle)and the tissues of these organs were prepared by cutting them in to pieces each with the same dimension .Each sample has fixed in the holder at a distance of 30 cm from CO_2 laser beam. The tissues exposed to CO_2 laser for 20 seconds with different power laser. The power and the spot diameter of laser was (5 to 10Watt) and (2mm) respectively, as shown in Fig.3.

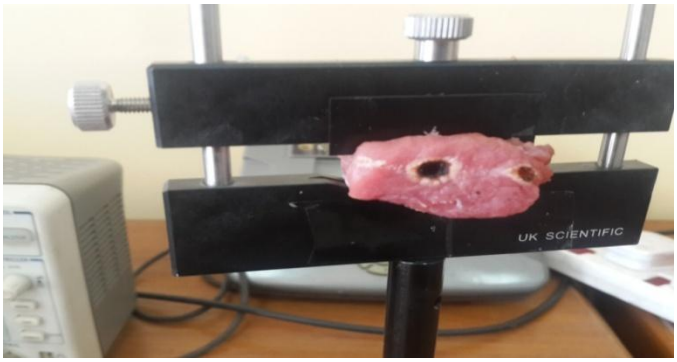

Figure 3:- Tissue fixed in the holder and shoot with CO_2 laser.

Then each tissue had been cut from the center of the spot in to two pieces to see the damage depth. Then each tissue was prepared for examination under light microscope by fixation of the tissue on glass slide using (paraffin) embedding section method.

Sample preparation:-

The samples are prepare after exposure to CW CO_2 laser by cutting samples into rectangular with dimension are (1 cm long, 0.5 cm width and thick) were put in formaldehyde 10% in as volume of 10 time as the sample size(v/w) [9] for 24 hr. After formalin, the samples placed in Ethyl alcohol with different Concentration to extract water from the samples. Then the samples were put in xylene for 2 hr. this step was repeated twice. Then the samples were put in molten paraffin wax for a day, and then put in the oven at a temperature 65°C. Next, the paraffin blocks are put on

ice to harden [9]. After the wax hardens, can make thin slices of the wax using a microtome. This instrument can cut slices just a few microns thick. Then , the wax in the samples are removed and the section is stained[15]. Finally, put the slides under the microscope, looking for thermal damage in tissue and determine it using scaled lens used on the microscope[16]. As shown in Fig.4.

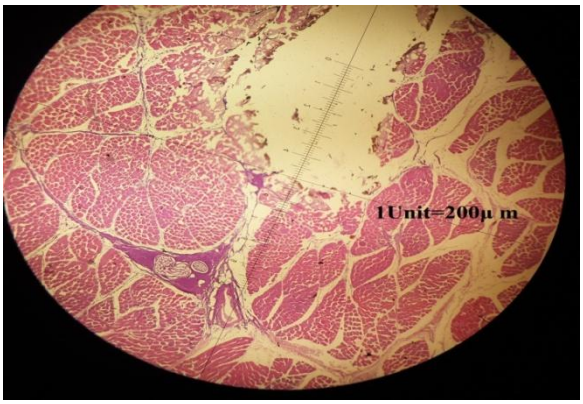

Figure 4:- The sample of musle tissue with power of 13w and time 20s under the microscope.

Result and Discussion:-

Measurements the percentage of water in different tissues:-

Different types of tissue are tested muscle, lung, liver, heart, brain and three type of tissues that has noticeable change in the percentage of water; which are used in this work muscle, lung, and brain ,as shown in table 1.

Table 1:- The water content of each tissue

Samples	Weight before dehydration	Weight after dehydration	Percentage of water %
Muscle	0.72	0.15	79
Lung	0.77	0.11	85
Liver	1.16	0.22	81
Heart	1.19	0.16	86
Brain	1.29	0.14	89

Measurements of damage depth in tissues:-

The thermal damage of tissue was seen using a microscope as shown in Figs.5, 7, 9 and the damage depth is measured using scaled optics used on the microscope, the result is as shown in table 2.

From Figs.6, 8, 10 and tables 2, 3, 4 it is found that the damage depth in tissue decrease as the CW CO_2 power (intensity) and tissue water content increase; assume all tissue is subjected to 20s of laser where quasi-steady state is insured.

Damage depth of muscle tissues:-

Table 2:- Theoretical and experimental damage depths of muscle tissue subjected to CO_2 laser.

Power (w)	Damage depth Dose (m)	Damage depth Arrhenius (m)	Damage depth (m) Exp.
5	464.23806×10^{-4}	4.95860093×10^{-4}	4.40×10^{-4}
7	3.04454420×10^{-4}	3.27241314×10^{-4}	3.0×10^{-4}
9	2.24680415×10^{-4}	2.4248862×10^{-4}	2.40×10^{-4}
10	2.0799612×10^{-4}	2.24112712×10^{-4}	2.30×10^{-4}
14	1.40291210×10^{-4}	1.5242065×10^{-4}	1.35×10^{-4}

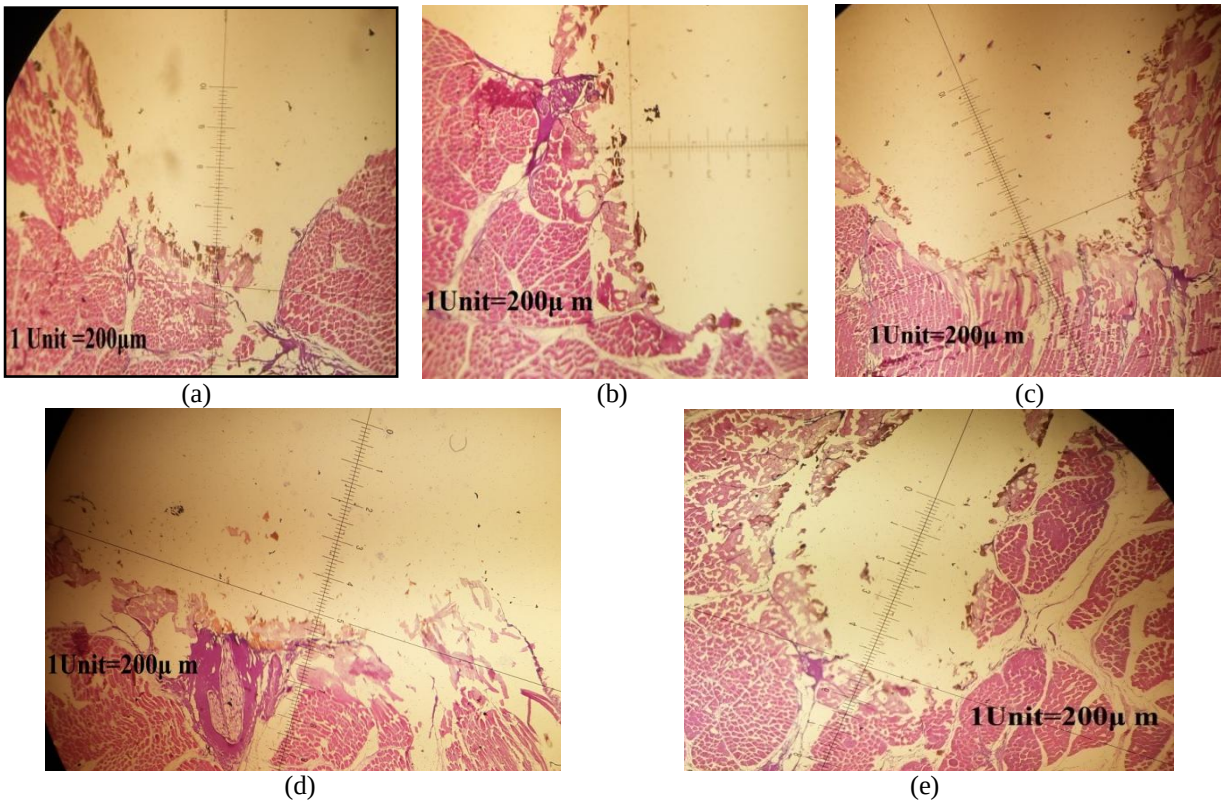

Figure 5:- Thermal damage in tissue of muscle under the microscope caused by CO_2 laser after20 s with power of :a) 5w, b) 7w, c) 10w, d) 9w, f) 14w .

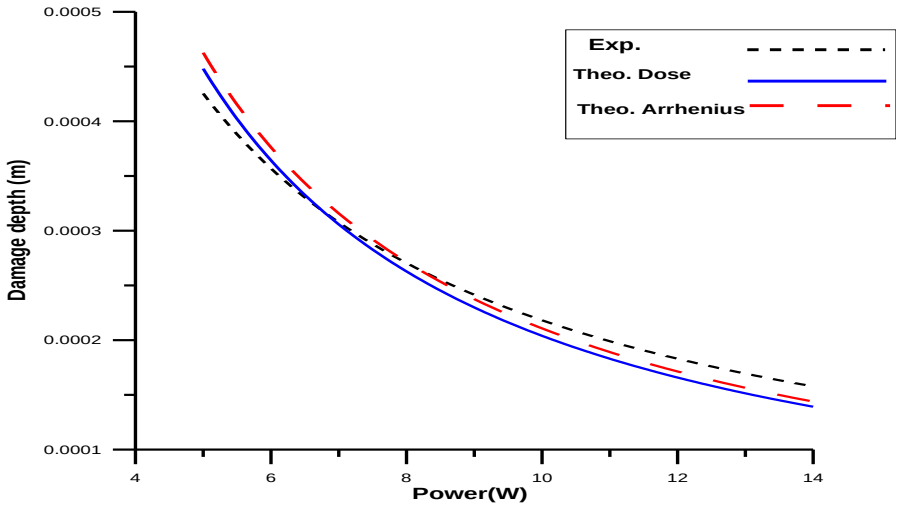

Figure 6:- The theoretical Dose, Arrhenius and experimental variation of damage depth with power in muscle tissue.

Damage depth of lung tissues:-

Table 3: -Theoretical and experimental damage depths of lung tissue.

Power(w)	Theoretical Damage depth Dose (m)	Theoretical Damage depth Arrhenius (m)	Exp.Damage depth (m)
5	3.877824×10^{-4}	3.8583245×10^{-4}	3.8×10^{-4}
7	2.6154004×10^{-4}	2.5961604×10^{-4}	2.5×10^{-4}
9	1.9554149×10^{-4}	1.9376549×10^{-4}	2.0×10^{-4}
10	1.67906706×10^{-4}	1.6576170×10^{-4}	1.60×10^{-4}
14	1.2589221×10^{-4}	1.2589221×10^{-4}	1.20×10^{-4}

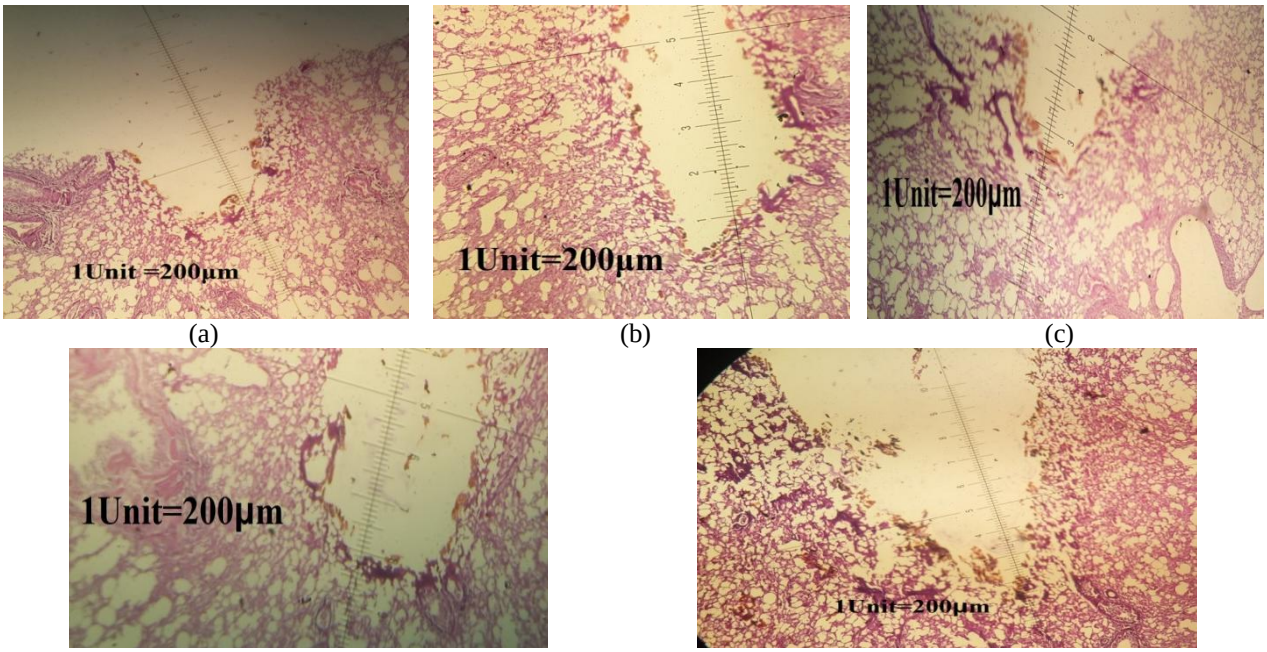

Figure 7:- Thermal damage in tissue of lung under the microscope caused by CO_2 laser after20 s with power of : a) 5w, b) 7w, c) 10w, d) 9w, e) 14w .

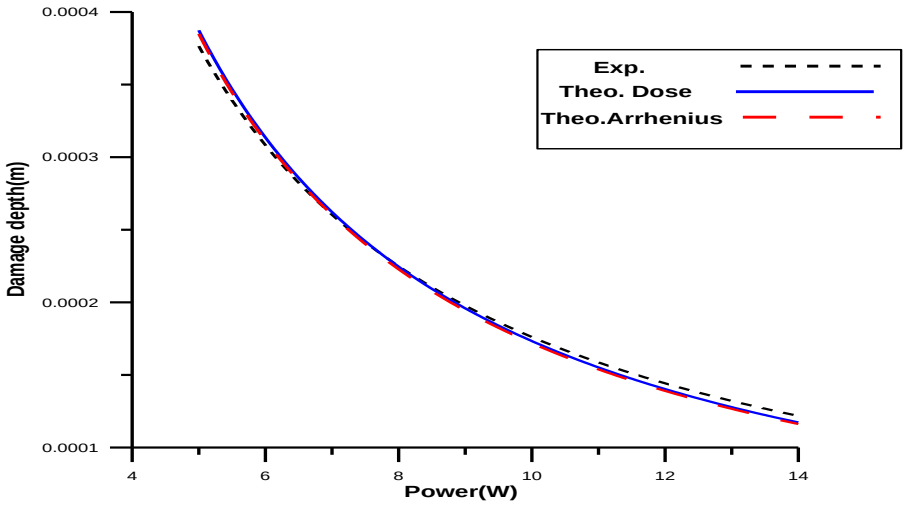

Figure 8:- The theoretical Dose, Arrhenius and experimental variation of damage depth with power in the tissue of lung.

Damage depth of brain tissues:-

Table 4:- Theoretical and experimental damage depths of brain tissue.

Power (w)	Theoretical Damage depth Dose (m)	Theoretical Damage depth Arrhenius (m)	Exp.Damage depth (m)
5	3.4903815×10^{-4}	3.6032215×10^{-4}	3.6×10^{-4}
7	2.2789054×10^{-4}	2.3682854×10^{-4}	2.4×10^{-4}
9	1.74672236×10^{-4}	1.820042×10^{-4}	1.90×10^{-4}
10	1.57619022×10^{-4}	1.6554202×10^{-4}	1.5×10^{-4}
14	1.0843271×10^{-4}	1.1123927×10^{-4}	9.8×10^{-5}

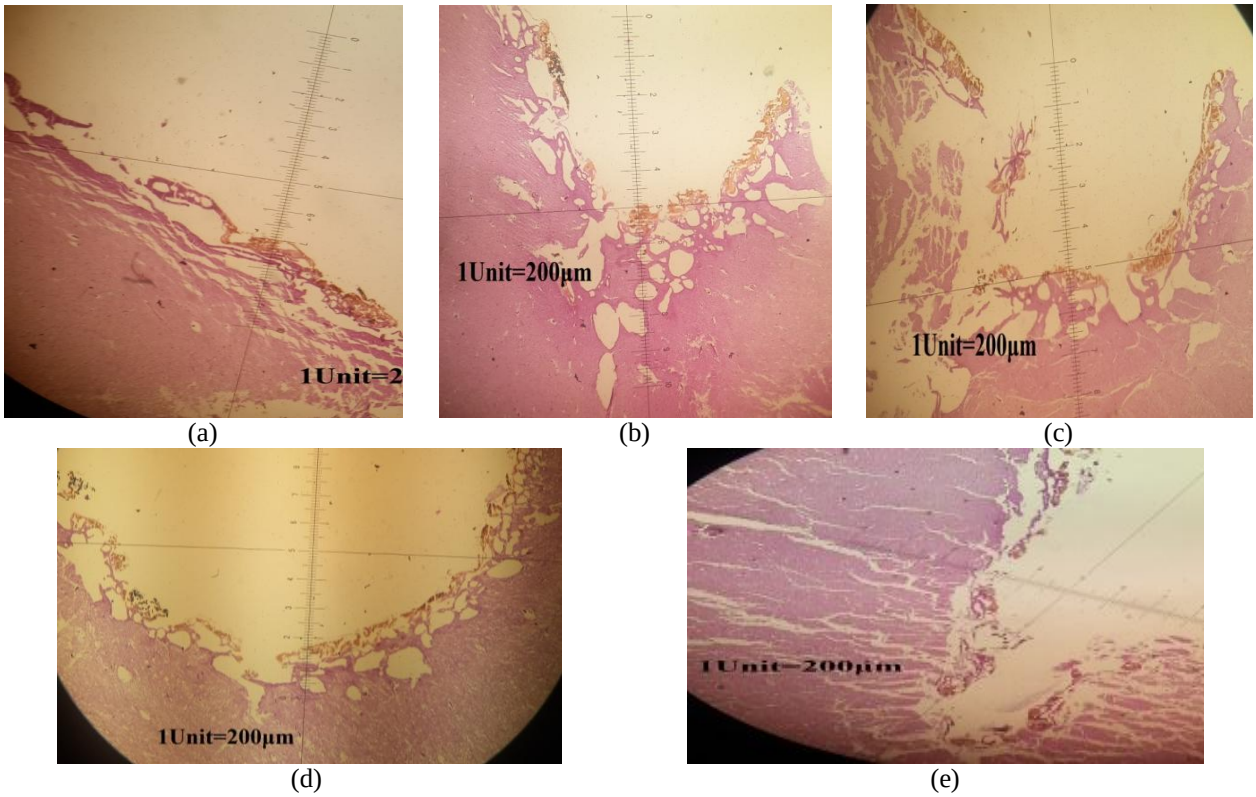

Figure 9:- Thermal damage in tissue of brain under the microscope caused by CO_2 laser after20 s with power of : a) 5w, b) 7w, c) 10w, d) 9w, e) 14w.

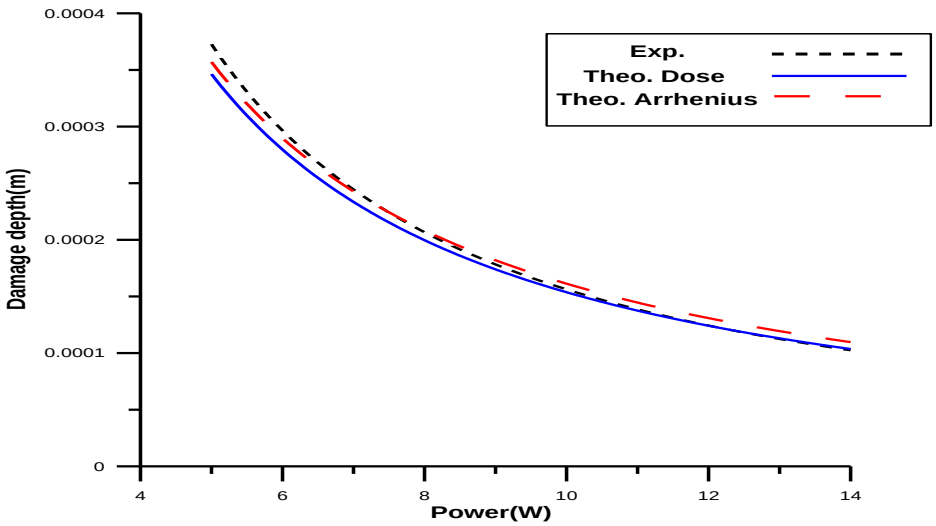

Figure 10: The theoretical Dose, Arrhenius and experimental variation of damage depth with power in the brain tissue.

Effect of water content on damage depth:-

Fig.11, shows that the damage depths of tissue having different water content, for muscle(79%),lung(85%),brain(89%),it is found that as power increased damage depth is decreased, also it is found that as water content increased damage depth is decreased. This is due to increase in cutting speed where heat is not allowed to accumulate to cause large damage.

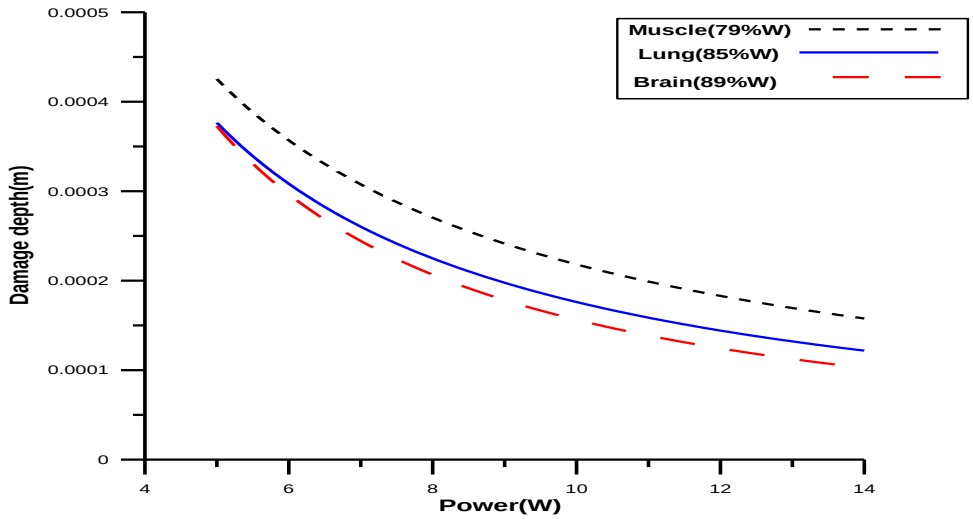

Figure 11:- The variation of laser power with damage depth at 20 s in the muscle, lung and brain.

The result of this work shows that the damage depth decreased as laser power intensity increased, which can be explained by that as power intensity increased ,the velocity of cutting is increased , so that a specific depth will have less time to accumulate heat that can cause damage .This explanation can also be applied to the reason behind decreasing damage depth as water content increased.
Lung tissue has less water content than brain tissues see table1, so that damage depth in lung is more than brain, while the brain has damage depth less than muscle and lung ,as shown in Figs.6, 8and 10.

The Arrhenius and CEM $_{43}$, models are the two most commonly used models that predicate thermal damage, as shown in tables 2, 3 and4.

The average error in obtaining thermal dose is found to be 7% for muscle, 2.6% for lung tissue and 5 %for brain tissue. The average error for Arrhenius is found to be 5.8% for muscle tissue, 2.8% for lung tissue and 5% for brain tissue. So that, Arrhenius equation is more accurate than thermal dose in obtain thermal damage depth.

The model of Arrhenius is enough to provide an accurate estimate of thermal damage and cell death. It is necessary in numerical model work to comprise several thermal damage processes operating in parallel to get a clear image of the probable result in tissues. Arrhenius model have that ability, while CEM_{43} does not have, as obtained by reference [17].

Conclusions:-

1. Water content in tissue has big effect on thermal damage depth, where as water content increases the damage depth decreases.
2. The damage depth of thermal damage can be calculated by two model of method, which is CEM_{43} and Arrhenius model. In addition, it is found that Arrhenius equation is more accurate than thermal dose in obtaining thermal damage depth, Comparing with theoretical and experimental finding.
3. It is found that as power intensity is increased, thermal damage is reduced but it is limited by phenomena of photo disruption.
4. CW CO_2 laser can be used successfully to cut or ablate bio- tissue, which result thermal damage.

Acknowledgment:-

The Authors would like to thank Department of Laser & Optoelectronic Eng., University of Technology, for the valuable collaboration and help.

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