Hunar Mustafa WASMAN

OPTICAL DETECTION OF OXIDATIVE STRESS ON CULTURED BLOOD SAMPLES TREATED WITH CCl_4

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by

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APPROVAL PAGE

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OPTICAL DETECTION OF OXIDATIVE STRESS ON CULTURED BLOOD SAMPLES TREATED WITH CCl_4

Hunar Mustafa WASMAN

M.S. Thesis – Biomedical engineering
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Thesis Supervisor: Assist. Prof. Dr. Haşim Özgür Tabakoğlu

ABSTRACT

There are many studies dealing with the measurement of oxidative stress in blood samples in vitro, nowadays there are different methods discovered for measurement of the levels of oxidative stress but because of their specifity biochemical methods have been accepted as golden standard. Biochemical measurement of MDA is one of specific methods for oxidative stress in different biological study area. Recently there are attempts to find new optical methods with more accurate measurements of oxidative stress. In the present study, light transmittances have been used to measure the influence of different concentrations of CCl_4 on cultured blood. In vitro exposure blood samples have divided into four groups, first and second groups were exposed to 1, 5 and 10 µmol of CCl_4, third group were exposed to 1, 3 and 5 µmol of CCl_4 while fourth group were exposed to 1 and 5 µmol of CCl_4. MDA levels of all groups of cultured blood samples were measured after exposing according to above concentrations of CCl_4 to insure effectiveness of CCl_4 on blood samples in vitro. The MDA elevations were not significant ($p>0.05$) except at 1 µmol of first group. Decrease levels of MDA were recorded at third group were significant only at 1 µmol CCl_4. Optical results showed increased light's absorption significantly at both 1 µmol and 10 µmol CCl_4 in first group. At second and fourth group, significant decreases of absorption were recorded at 1 µmol CCl_4. Light absorption increased at 1 and 3 µmol CCl_4 of third group but only at 1 µmol were significant. There were no any significant differences between different concentrations at all groups with their controls at any wavelength.

Keywords: Oxidative stress, MDA, Optical Detection, Blood Culture.

CCl_4 İLE İŞLEME TÂBÎ TUTULMUŞ KAN ÖRNEKLERİNDE MEYDANA GELEN OKSİDATİV STRESİN OPTİK ALGILANMASI

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ÖZ

Kan örneklerinden oksidativ stresin ölçülmesi ile ilgili birçok çalışma vardır. Günümüzde oksidativ stres miktarının belirlenmesi için farklı yöntemler keşfedilmekte olsa da yüksek seçiciliğinden dolayı biyokimyasal yöntemler altın standart olarak kabul edilmektedir. Oksidativ stresin belirlenmesi amaçlı MDA miktarının biyokimyasal yöntemler ile ölçümü farklı biyolojik alanlarda kullanılmaktadır. Oksidativ stresin tayininde yeni optik yöntemlerin bulunması için yapılan çalışmalar oldukça yenidir. Bu çalışmada kan kültürlerine uygulanan farklı konsantrasyonlardaki CCl_4 ün etkisinin ışık geçirgenliğine etkisi araştırılmıştır. Başlıca 4 grup oluşturulmuştur. Birinci ve ikinci gruplarda 1, 5, 10 µmol CCl_4 kullanılmıştır. Üçüncü grupta 1, 3, 5, µmol CCl_4 kullanılmıştır. Dördüncü grupta 1 ve 5 µmol CCl_4 kullanılmıştır. Yukarıda belirtilen miktarda CCl_4 uygulamasında sonra MDA ölçümleri yapılmıştır. İlk grupta yer alan 1 µmol uygulaması dışında anlamlı bir fark bulunamamıştır. Üçüncü grupta genel bir düşüş olmasına rağmen yine sadece 1 µmol uygulamasında anlamlı fark tespit edilmiştir. Optik ölçümlerde elde edilen sonuçlarda ilk grupta yer alan 1 ve 10 µmol lük uygulamalarda anlamlı bir ışık soğrulması tespit edilmiştir. İkinci ve dördüncü gruplarda 1 µmol lük uygulamada soğrulmanın anlamlı bir şekilde azaldığı tespit edilmiştir. Üçüncü grupta 1 ve 3 µmol lük uygulamalarda ışık soğurulması artsa da sadece 1 µmol lük uygulamada anlamlı bir fark tespit edilmiştir. Çalışma grupları ve kontrolleri arasında yapılan karşılaştırmada herhangi bir dalgaboyunda anlamlı bir fark tespit edilmemiştir.

Anahtar Kelimeler: Oksidativ stres, MDA, Optik Algılama, Kan kül

To all members of my family

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LIST OF SYMBOLS AND ABBREVIATIONS

SYMBOL/ABBREVIATION

MDA	methylene dianiline
ROS	reactive oxygen species
DNA	deoxy ribonuclie acid
RS	reactive species
RNS	reactive nitrogen species
UV	ultraviolet
ALD	alcoholic liver disease
OxS	chronic cerebral oxidation stress
AST	aspartatw transaminase
ALT	alanin transaminase
ALP	alkaline phosphatase
CCL4	carbon tetrachloride
IL-1b	inter-leukin-1b
TNF-a	tumor necrosis factor-a
IL-4	tumor necrosis factor-4
IL-12	inter-leukin-12
SOD	superoxide dismutase
GPX	glutathione peroxidase
CAT	cutalase
GSH	glutathione
IR	infrared
PHA	phytohaemaglutinin
FBS	fetal borine serum
SAC	sample chamber
NIR	near infrared
VIS	visible

CHAPTER 1

AIM OF THE STUDY

The study aimed to find out the ability and use of light in different wavelengths to detect the biochemical changes of blood samples after inducing to oxidant agents. These were achieved by (absorption/reflection) of the light ranging from (450-1100 nm) which includes to regions (NIR, VISIBLE). Evaluation the absorption rate with the classical methods for detecting oxidative stress in blood sample may give clues to finds a new or more accurate method to find oxidative stress in blood samples.

Motivation:

This study may give some beneficial information about the uses of biomedical apparatuses and its application to detect some biochemical changes of blood sample while expose to toxicant agents. Absorption/reflection of light gives information at ranging wavelength from (450-1100 nm). Most useful of biochemical sensor or optical methods are preferable because of their specific properties such as easy in use and operation , sensitive and gives an accurate results. Wide range of uses of the optical sensor for analytical chemistry and composition of chemicals of different kinds of samples. The interest compound can interact with light without need to prior modification of chemical.

CHAPTER 2

INTRODUCTION

Laser applications and different spectroscopic devices for diagnosing soluble and non soluble particles, molecules as well as cell and tissues are investigated and designed with their different principal of works [1]. Spectroscopes are designed to detect or to diagnose of variety of disease in biomedical and clinical area, most of the spectroscopic applications are suitable to detect according to kinds of sample or its composition so that the powerful technique for analysis and measure depend on the complex composition of biological samples [2]. The potential of most spectroscopy comes from the ability of diagnosis the biochemical changes due to any physiological changes or as are results of infections [3]. Advantages of some kinds of spectroscopy such as Raman Spectroscopy for biological and medical have high specificity and ability to obtain information with no stain or reliable requires [4].

At the center of the subject of potential oxidation are free radicals step with interactive oxygen and nitrogen classes, the oxidants are the collection of the all mentioned. The oxidants are very small particles if compare to the molecules like proteins, but some oxidants has a molecular weight less than 100 micrograms. The bizarre electron number of chemicals makes them to become a free radical. The small molecules / ions which are able to react even with a few amount of activation energy and with instant lifespan; this is as considered in term of oxidative stress. Most of these molecules/ions can enter and out through the plasma membrane because of their very tiny size. As a subclass of both oxygen and nitrogen the free radicals work and are the able reactant. Mitochondria are place to create the main portion of reactant classes such as oxygen with the help of metabolic aero reactions. A section of the O_2 production technique as a superoxide anion is performed in the internal layer of mitochondrial membrane, which leads to convert O_2 to H_2O [5]. The production of reactive oxygen

Species (ROS) becomes more capable than the antioxidant triggers to which oxidative stress happens. In this situation many various types of incorrect oxidative contents of the cell occur and trigger to fewer faults such as the diseases that are related to the psychiatrics. The manufacture of ROS can dominate the brain immune system because of the psychiatric diseases which detect this state can be detected from a proof of clinical and preclinical situation. In some neuropsychiatric diseases such as autism, depression, schizophrenia and anxiety pathologically can be affected by the damage of oxidation. [6].

The biological particles and physiological conditions all membranous lipid, proteins including DNA and other synthetic proteins are arbitrated by oxidation results in many disorder and diseases. Dysfunctions and structural changes of cell membrane by binds free radicals attached to lipids' compositions are and win over of lipid per oxidation leads structural fine and disturbances [7]. There are attempt to diagnose more about free radical and bimolecular reactions and their biological changes and also types of disease inductions, the common most researches are on reactive species (RS), however it is difficult to imagine the exact pathways figure due to some reasons. Such as: (i) involvement of reactive species and high diversity among specific reactions. (ii) Stability rates are very low and high reactivity, or tend to steady soon. (iii) according to organism's physiological state, and (iv) no suitable technical tools for evaluation of absolute rate and even nearly reasonable levels [8].

In the last twenty years, there has been hug attempt to use of free oxygen and nitrogen bases, commonly known as "reactive oxygen species," (ROS) and "reactive nitrogen species" (RNS) and their effects physiological and clinical medicine. ROS and RNS are produces from many sources and factors like physical such as : induce by X-rays, UV light and gamma rays irradiations. Chemical factors like production of metal-catalyzed reactions became the result of radiant chemicals and ion free chemicals. Also there are biological factors are produced by neutrophils and macrophages during inflammation; products of mitochondria-catalyzed electron transport reactions and other mechanisms [9]. Reactive oxygen species (ROS) are series of reactions which includes unpaired electrons and include singlet hydroxyl radicals and oxygen free radicals. ROS are an essential component of pathways signal of cell signaling. Therefore, instability conditions due to free electron or unpaired electron cells, make electrons get high

receiving rate with cells' membrane components that occasionally generate their damaging finally. The rate of free radicals in the body has different and significant evidence caused endothelial injury, cardiovascular dysfunction and diseases with progression of the ROS [10]. The most reactive oxygen radical is hydroxyl radical which living arrangement by Fenton reaction. Which are 9- 10 seconds were calculated as its half-life in living cells, so that, direct measurements are difficult by traditional analytical techniques and it requires very high sensitive and specific equipment. Generally oxygen radicals and hydroxyl radicals are byproduct of oxidative metabolism which are harmful, by reacts with the membranous molecules and other cellular particles resulting in damaging them in living systems, and also, in the other hand, the beneficial of free radicals in damaging ingesting molecules in some microorganisms like bacteria through dispersion of the engulfed molecules [11].

Every days exposure of free radicals inductors are surrounded or deals with such as cigarette smoke and alcohols which have tendency to stimulate and known to have reactive oxygen species (ROS) creators and results in oxidative stress [12]. There are many factors causes of chronic cerebral oxidative stress (OxS), cigarette smoke is one of them which is contains mixture of burning products most approximate 5000 metals among of them there are high number of carcinogenic and toxic. Cerebral OxS as its name come from causes and leads operationally and also uses as detections to damage brain's tissue (e.g., lipid per oxidation, proteolysis) which resulting from cause by reactive oxygen species (ROS) and reactive nitrogen species (RNS), and extra oxidizing agents. Cigarette smokes components after burning mostly produces extremely huge amounts of free radicals in both form (short-and-long-lived free radicals) and in contrasts smoking have inhibition properties for synthesis of essential intracellular anti-oxidants, such as glutathione [13, 14].

There are good knowledge of free radicals are typically have critical roles in several diseases conditions such as several malignant disease, disordering of cardiovascular, inflammation and arthritis and other diseases in different organs. Chemicals such as carbon tetrachloride make breakdowns of complex molecules in living organisms via radicals induces lipid per oxidation, membrane disorder and damage of liver cells and organelles, resulting in necrosis of hepatocytes and swelling which finally leads to release AST, ALP and ALT cytosolic enzymes into the

circulating blood [15]. The seek to discover and blocks or prevents effects of free radicals to damage and causing different diseases through naturally occurring antioxidants has become a great challenges over the last few decades. CCl_4 is traditionally used to induce organic toxicity in vitro/in vivo models [16].

CCl_4 is a classical cellular toxicant leads to induce oxidative stress mediated cellular toxicity. Inactivation of CCl_4 molecules comes from converting to trichloromethyl free radical under control of cytochrome P450 isozymes. So that free radicals of trichloromethyl interacts with oxygen and become highly toxic, reactive trichloromethylperoxy radical. Polyunsaturated fatty acids of cell membrane will be predatory of final products of free radicals of trichloromethylperoxy, attacking of lipid membranes are continue to chain reaction causing in damaging of membrane structure, disordering processing of energy of cell and synthesis of protein [17].

Effects of free radicals leads to change different other criteria in the body, but also prompt the over expression of mediatory factors of inflammatory such as (interleukin-1b [IL-1b], tumor necrosis factor-a [TNF-a], IL-4, and IL-12) through stimulating nuclear factor-kB (NF-kB). Mentioned mediators then stimulate neutrophils, which are inflammatory cells, prompting to different cell deaths mainly liver cell death [18]. Experimental animals exposed to CCl_4 shows good results in activation of antioxidant via testing lipid per oxidation in different cell lines especially hepatic damages [19].

Oxidative stress are generated not only from exposure of chemicals, physicals and biological factors but also intense exercise activity and any metabolically changes with associated emotional stress are resulting in oxidative stresses, so that production of byproduct of chemicals of body take places such as free radicals and cortisol. According to byproducts the drain produced by body systems. Byproducts chemicals have a core roles in damaging of the cells and tissues, due to breakdown of tissues and cells of tissues by mentioned byproducts it called catabolic. This not conceders as an organic chemistry class, because they are not participate to build up any organic matters in the body or they are not contribute in components of any tissues and cells but its supposed that they participate in production of energy or have a vital role in the processes of

generation of energy requires for intense exercises, but in the other hands bad byproducts leads to induce oxidative stresses. Because of limited anti productions to normalize or neutralize those oxidative stresses factors by the body, the body tend to have dependable clearance agents, and because of this limitations, oxidative stresses agents or mixture gets effect on the tissue negatively and may cause tissue damages and/or nucleic matter damages and performance progressive declines, imbalance in the rate of produced antioxidant by body with the rate of free radicals may have an effects on immune system schemes, in this way those over exposure to free radicals may lead to generate cancer or in somehow consider as a carcinogenic [20].

Failure of organ or tissue comes from the toxic damages of the cells, according to the power of toxicant agent and the number of free radical rate , the number of damaged cells are increased resulting the organisms death. That's why in pharmacological field it is most important to check a toxicity of produced drags and also checking free radicals induced by produced drags .(in this case toxicity include both biochemical and cellular toxicity) . Due to the nature of cell variability in the body, cell deaths are standing on the changes accruing inside and or outside (surrounding) of the cells or tissue such as biochemical changes all cells of body tissues of few of them are damaged without touched to the toxicant agents actually to the membrane or chemical compounds of the cell. Temperature and radiation in general have damages cells through coagulation to cells content , so that in this case there no any specific chemicals or toxic chemical participating in damaging of tissue or they consider as physical agents . some of oxidative stress are come from reluctant supply of nutrient especially reduce body income of glucoses of oxygen may cause inactivate or passive in the activity for producing sufficient material for tolerance the other main sources for generation as oxidative stress are xenobiotice substances . There are several examples on the interactions of biochemical out of causing observable damage of cells or there organelles such as:

Exchanging of carbon monoxide with oxygen in hemoglobin molecule is a toxic chemical process, which is called replacement molecules.

Some xenobiotic agents can interfere with somebody chemical and leads to induce transmit a messages like what is occur in synapses for example: acetyl cholinesterase enzyme are reserve by organophosphate pesticides.

It is general human bodies have very complex organisms which consist of over 200 different types of cell. Distributed by their mission to different tissue each cell types have their function in the tissue is very important any changes accrued to normal function of tissue lead to hug disorder in other organs systems. [21].

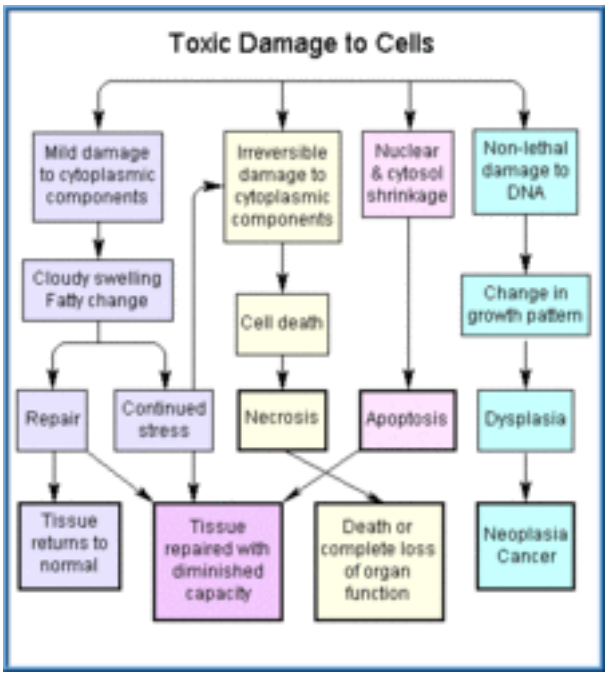

Figure 2.1 Toxic damage to cell [22].

Organisms caused by exposure to different or mixture sources of free radicals induction produce series of defense mechanisms. Mechanism Defenses to free radical leads to induce oxidative stress includes: (i) repair of the mechanisms (ii) protective mechanisms (iii) antioxidant championship and (iv) corporal defenses. There two types of antioxidant defenses which are; Enzymatic and non enzymatic antioxidant defenses.

The enzymatic one involves catalyses (CAT), glutathione peroxides (GPx), superoxide dismutase (SOD). Non-enzymatic antioxidants are includes, tocopherol (Vitamin E), ascorbic acid (Vitamin C), carotenoids, glutathione (GSH), flavonoids, and some extra antioxidants. Under normal situations, there are balance between both the intracellular levels of above antioxidants and the activities. This equilibrium is important for the proliferation of organisms and their health [23]

Kind of defenses of antioxidant

1. There are some kind of antioxidants gives or donate one of its electrons to react with unstable free radicals to neutralize it this called primary antioxidant or scavenger antioxidants.

2. there are some possibility of act mechanisms of antioxidant called secondary or preventive antioxidants, this possibility includes;

a) Participate of metal catalyzed reaction are not allowed through detention of transition metal ions.

b) Production of ROS by reaction of metal ion transition with glutathione peroxidase and catalase can remove the peroxides.

c) ROS removal etc.

3. There are processes called repair, this process can remove bimolecular which they are damaged before accumulation e.g. enzyme methionine sulphaoxide reeducates can repair damaged DNA [24].

Nowadays there are new achievement and ongoing of the applications for the detection of the biochemical reaction. Detection of the toxins and identification of low concentrations with high affinity or detection of biochemical changes as a result of toxicant agents [25]

Biochemical defectors such as raman spectroscopy one of the powerful instrument for detection of cell behavior, metabolism changes, cell death or cell lines and effects of toxicant agent that lead to change biochemically. Most of the spectrscopical applications one uses in vitro by preparing solution or reaction in the lab [26]. The power of the detect ability of the different spectroscopical device depends on the principle of works, the spectrum and the type of detectors for detecting the rate of high transmission and also lights which scattered while exposed to samples [27]

The powerful and useful spectroscopy uses for detection of biochemical concentration in low rates zolix device which have different types but the principal works of mentioned of light produced in light source of zolix can produce (UV,VIS and NIR) wave length which are useful for detection of the amount of particles or chemical compounds of in sample medium those different kinds of wavelength can be used separately or together by setting a program of device [28].

Wavelengths of UV ranges (10-400nm), VIS wavelength ranges are (400-767nm) and NIR wavelength are (767-3200nm). So that according to the nature and type of biochemical agent or reactions the selection of ranges of the wavelength will sets the rate of light scattered, transmitted or reflected are defects by special detector which is good enough sensitive to lights can separate and distinguish between every single nanometers transmitted or scattered from sample media [29].

CHAPTER 3

MATERIAL AND METHOD

3.1 Absorption / Transmission/ Reflection Spectrophotometer

ZLX-AS system has a wide range of application with the high performance of absorptions with the good quality of measurements, this system is useful for both solid and liquid samples transmissions and reflection.

This system has a specific light source of monochromator which has ability to produce and ranging the light wavelength in UV to IR. High affinity and of this system can be obtained by its light source. Detection and of absorbance light by specific detector makes this system more efficient which cannot detect easily by other conventional spectrophotometers. The system has a side and foreword light detectors that give other properties that can integrate optical sphere that make it measurable.

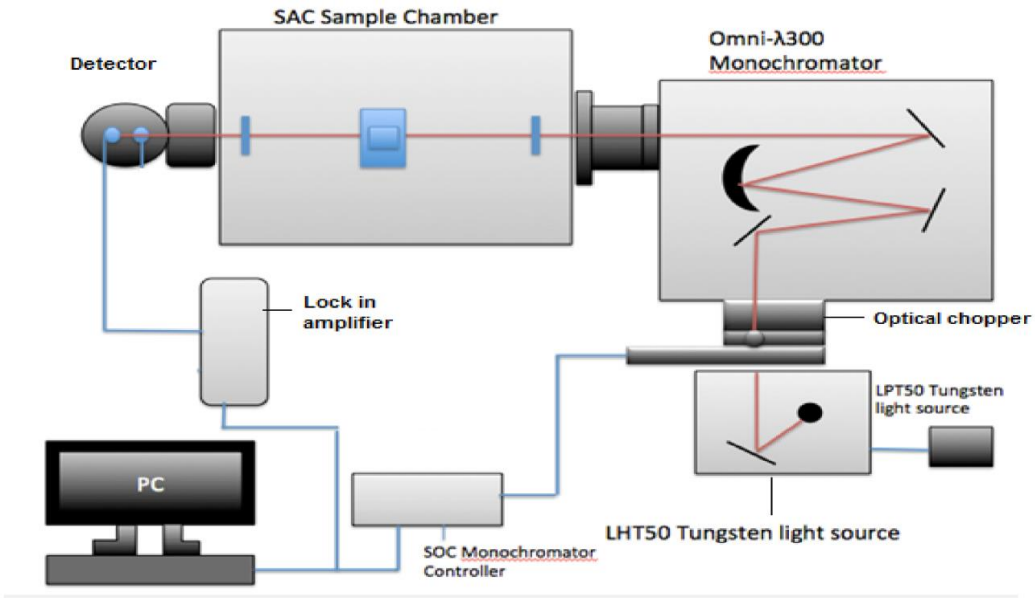

Figure 3.1 Highlight of Zolix device shows all parts[30]

3.2 Monochromator

Device has excellent optic performance, by multipurpose monochromator which apply to the system by arranging from UV to IR with good resolution. It is very sensitive fully automatic system from 300 nm.

3.3 Champer sample

Samples are evaluated by SAC, those used for application for measuring, absorption, reflecting and transmission. The SAC is useful for converges the beam while passing through sample and make beam paralleled by containing different quarts lens. Parallel beam was used or applied to make light easier for measuring absorption, reflection and transmission, in the other hand the converge beam is applying for measuring reflection, absorption and density of liquid or gas sample. The application of quarts lens for focusing beam with turns 360 degree properly of sample[31].

3.4 Osi silicon detector

There are different types of detector used in zolex device and each type is used for specific and different wavelength. One of the most sensitive detectors is silicon detectors, the range of such detector UV is between 200-1300 nm. The composition of silicon detector of Dsi 200 built in UV sensitive Si detector. For low dark current detector is Dsi 300, and 1-1 amplifier for the current to voltage conversion. Specification of the detectors is shown in the table below.

Table 3.1 Specification of detector [32].

SPECIFICATION OF DETECTOR	DSi200 (UV sensitive) Import UV Enhanced
Effective receiving Area (mm^2)	100(Φ11.28)
Spectral range (nm)	200-1100
Peak wavelength (nm)	-------
Peak Responsivity (A/W)	0.52
Response @254nm (A/W)	0.14(>0.09)
Response time (μs)	5.9
Operating Temperature (℃)	-10～+60
Storage Temperature(℃)	-20～+70
Shunt resistor R_{SH}(MΩ)	10(>5)
Noise equivalent power NEP (W/√Hz)	4.5×10^{-13}
Dark current (25℃ ; -1V)	-------
Junction capacitance (pf)	4500
Signal output	Current
Output signal polarity	P

3.5 Tungsten–Halogen light source

Spectral range of wavelength are 300-2500 nm and has about 400 hours long life for zolix light [33].

3.6 Lock in amplifier

Little nano-volts are very small signals. They are detectable by using lock in amplifier technique. For detection of signal out of phase component and specific reference is called phase–sensitive detection. Signals such as noise signals is unacceptable or an measurement of frequencies other than reference frequency. Phase locked Loop mostly generates internal signals and this lead to generate external reference of lock in amplifier[34].

3.7 Optical chopper SR540

Modulations of the signal are made in optical chopper SR540 which single and double beam are measured. Analogue voltage frequency control, front panel frequency control, and two outputs, frequency with selectable operation mode are four digital frequencies of optical chopper SR540. Aluminum is composed of blades that provides for two different states with different frequencies. 5/6 slot blades are supplied for up to 400 Hz. 25/30 slot blades are supplied for up to 3.7 frequencies.

Figure 3.2 Absorption–reflection spectrophotometer

Above figure shows the parts of Zolix instrument which used for study. Whereas:

A. Light source

B. Optical chopper

C. Monochromator

D. Sample chamber

E. Detector

3.8 Instrumental protocol

Adjusting the light frequency of zolix device is prepared by filtering the light which is (223 Hz) of the optical chopper part of the device and light separation of monochromater having three different grades. Choosing sufficient grade is the most important step which is grade two, the light will adjust from (450-1100 nm) by outstanding light on grading system, and the light goes to chamber part, so the part of light will scatter and the other part will transmit through testing samples.

On the covert transmitted scattered light is detected by special detectors. After adjusting the light, only light of the zolix device was measured and then only cuvette was used or empty cuvette to insure adjustment of light and also fully transmission of cuvette.

figure 3.3 Optical Chopper SR540 [35]

3.9 Experimental procedures

3.9.1 General procedure

1) Collection of blood sample and putting in four different heparinized tubes. So as to each tube contains (2 ml) of blood sample. The samples were taken from young healthy male, non smoker. average ages from 20-28 years old.

2) Preparations of four different concentrations of CCl_4 which were (1, 3, 5 and 10 µmol)

3) Induce each concentration to each different cultured blood so that first and second groups were included (1, 5, 10 µmol and control), third group were includes (1, 3, 5 µmol and control) and the forth group was (1, 5 µmol and control).
4) Estimation of the MDA level of each groups and comparisons to their control to insure that CCl_4 induced oxidative stress.
5) Sufficient grade for setting wave length of light is grade two and adjust the wavelength from (450 – 1100 nm). The wave lengths were adjusted to increase 5 nm in the scanning steps are 5 nm.
6) Setting optical chopper : 233 H2, light power 6.45 ampere (A).
7) recording and analyzing the results to comparing each CCl_4 concentration to controls.

3.9.2 Preparation and Storage of Lymphocyte Culture

Lymphocyte medium was set up by adding; RPMI medium, L-glutamine, PS (1% penicillin-streptomycin), PHA (1% Phytohaemaglutinin) and FBS. Solution were prepared in (8 ml) of sterile cell culture tubes were shared. All cultures were maintained in darkness. Then they were stored (-20 ºC) until use. Lymphocyte cultures were setted up by adding (0.5 ml) of heparinized whole blood to RPMI-1640 chromosome medium supplemented with 15% heat-inactivated fetal calf bovine serum (FBS), 100 IU/ml streptomycin, 100 IU/ml penicillin, and 1% L glutamine. Lymphocytes were stimulated to divide by 1% Phytohemaglutinin. CCl_4 (in a concentrations 1,3,5, 10 µmol according to mentioned groups) were added to the cultures just before incubation. CCl_4 was used as an oxidative genotoxicant to test the anti-genotoxic and anti-oxidant potency [36]

Figure 3.4 RPMI Media

3.9.3 Biochemical analysis

The cell homogenates were prepared at a 1:10 (w:v) dilution in (10 mm) potassium phosphate buffer, pH 7.4. Samples were centrifuged at (3000 rpm) for (10 min) at (4 °C), and the supernatants were collected and immediately assayed for enzyme activities. All samples were measured in six fold [37]

3.9.4 MDA Assay

MDA levels in the cell culture supernatant were determined spectrophotometrically according to the method described by Ohkawa, et al. (1979). A mixture of 8.1% sodium dodecyl. sulphate, 20% acetic acid, 0.9% thiobarbituric acid was added to 0.2 ml of sample, and distilled water was added to the mixture to bring the total volume up to (4 ml). This mixture was incubated at (95 °C) for (1h). After incubation, the tubes were left to cool under cold water and (1 ml) distilled water with (5 ml) n-butanol/pyridine (15:1, v/v) was added, followed by mixing up. The samples were centrifuged at (4000 xg) for (10min). The supernatants were removed, and absorbance were measured with respect to a blank at (532 nm). 1,1,3,3-Tetraethoxypropane was used as the standard. Lipid peroxide levels were expressed as μmol/L MDA. All photometrical measurements were performed with an ELISA micro plate reader [38]

Figure 3.5 ELISA system (microplate reader, power supplier and computer system)

Figure 3.6 Universal micro plate reader model ELx 800

3.10 Data analysis

Most of the biological and biomedical research has special data. According to the types of data, the researcher should choose sufficient tests to testing hypothesis, accordingly student t-test which is a part t-test was chosen.

Choosing mentioned statistic test to observe the change between sample while treated CCl_4 in different concentration on the oxidative stress level of the blood and also absorbance level of the sera. To answer whether there is any significant difference between studied groups by getting p-value. From t-test if p-value has less than 5% that means there no significant difference between studied group and vis-versa [39]

CHAPTER 4

RESULTS AND DISCUSSION

This study sheds light on the effects of CCl_4 in different concentration to induce oxidative stresses so that MDA level of sera was studied in all concentrations. Then application of Zolix instruments light to observes any difference in absorption of sera after adding CCl_4 at different concentrations and comparing to control.

Table below shows MDA level of sera, in different concentration of CCl_4. There are many studies mentioned the effect of CCl_4 to induce oxidative stresses and their effects were lead to elevate the level of MDA. But there is less study that exposed different concentration to blood cells in vivo[40].

Table 4.1 Mean and t-test of MDA levels for each concentration of CCl_4 added to the groups of cultured blood.

MDA measurements		Concentrations of CCl_4				
		Control	1 μmol	3 μmol	5 μmol	10 μmol
First group	Average	37,01923077	53,36538	-	46,15385	53,20512821
	T test		3,198E-06*	-	0,000468469	0,000115567
Second group	Average	49,51923077	98,23717949	-	66,66666667	53,20512821
	T test		0,013868329	-	0,00092445	0,02500736
Third group	Average	133,7606838	108,974359	92,30769231	116,2393162	-
	T test		8,416E-06**	1,08132E-06	0,011114706	-
Fourth group	Average	104,7008547	172,6495726	-	90,5982906	-
	T test		0,211236161	-	0,519003584	-

In the first group the average MDA levels were increased, but only at 1 μmol CCl_4 concentration is significant ($p<0.05$).

There are also increasing in the average levels of MDA in second group but the changes were not significant at any concentrations ($p>0.05$).

The average MDA levels in third group was decreased at all concentrations (1 **μ**mol, 3 **μ**mol and 5 **μ**mol) the decreasing were only at (1 **μ**mol) CCl_4 were significantly different ($p<0.05$) while at (3 **μ**mol and 5 **μ**mol) CCl_4 were not significant ($p>0.05$).

In the fourth group, there were increasing at (1 **μ**mol) CCl_4 but decreased at (5 **μ**mol) CCl_4, in both concentrations the average levels of MDA not significant ($p>0.05$).

At low concentration of CCl_4 blood cells are more active and trys to neutralize the effect of CCl_4 on the blood cells. With the increase of CCl_4 concentrations and the time of exposure blood cells start damaging, so that with the increasing of CCl_4 concentrations the level of MDA start decreasing [41]. In this study there are elevation and decreasing of the level of MDA at four groups were different this may because of damaging red blood cells causes by CCl_4 in different rate.

In this case study exposure of blood cells in vivo to CCl_4 with different concentration repeatedly had led to increase in the mean of absorption of zolix. The first group as shown in table (3.2), there were increasing on the average of absorbance of concentrations of CCl_4 (1 **μ**mol, 5 **μ**mol and 10 **μ**mol) comparing to control, this increment was significant in both (1 **μ**mol and 10 **μ**mol) CCl_4 concentration ($p<0.05$) but increment were not significant in concentration of (5 **μ**mol CCl_4) ($p>0.05$).

Table 4.2 Mean and t-test for each concentration of CCl_4 added to the groups of cultured blood at different wavelength starting from 400 nm ending with 1100 nm.

Zolix absorption measurements		Concentrations of CCl_4				
		Control	1 **μ**mol	3 **μ**mol	5 **μ**mol	10 **μ**mol
First group	Average	0,013480608	0,020986968	-	0,020055037	0,018403674
	T test		0,105667789	-	0,003165352	5,3376E-08*
Second group	Average	0,022271157	0,021698445	-	0,019792247	0,018962758
	T test		7,4950E-09*	-	2,4505E-07*	1,21506E-07
Third group	Average	0,011703494	0,011810813	0,011864564	0,011586844	-
	T test		2,4624E-25*	1,44399E-07	7,1998E-08*	-
Fourth group	Average	0,003494898	0,003464047	-	0,003271301	-
	T test		5,2025E-06*	-	1,75571E-07	-

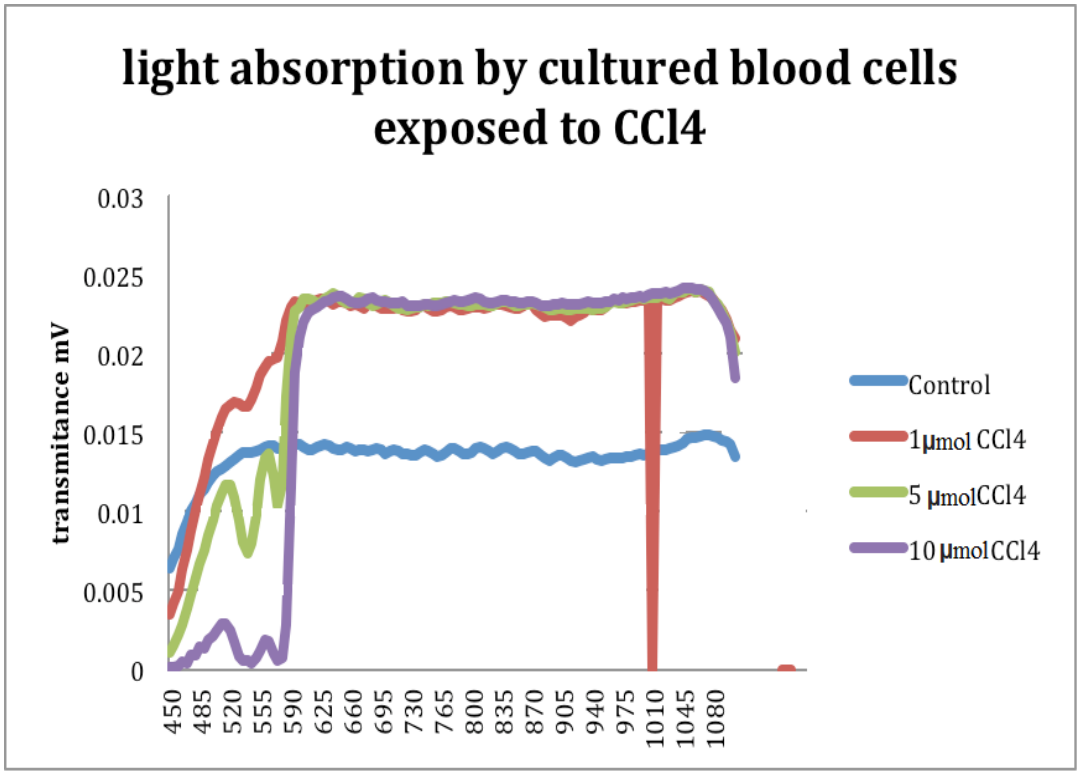

Figure 4.1 Optical response of the first group of effects of different concentrations of CCl_4 in cultured blood cells to the light spectrum from 450 nm to 1100 nm.

Absorption was similar at NIR region at all concentration of CCl_4 but the difference was in VIS region. The difference in the absorbance of first group of cultured blood show that with the increase of the concentrations of CCl_4, light transmission were decreased means transition of light at VIS region of 1 µmol CCl_4 were higher than 5 µmol CCl_4 and also the light transmission at 5 µmol CCl_4 were higher than 10 µmol CCl_4. At both region (NIR, VIS) average of light absorption were increased but only at 10 µmol were significantly different ($p<0.05$).

In the second group the average of absorbance was significantly decreased in both concentration (1 µmol and 5 µmol) CCl_4 ($p<0.05$). While decrement were not significant at (10 µmol) CCl_4 ($p>0.05$) as shown in (table 3.2).

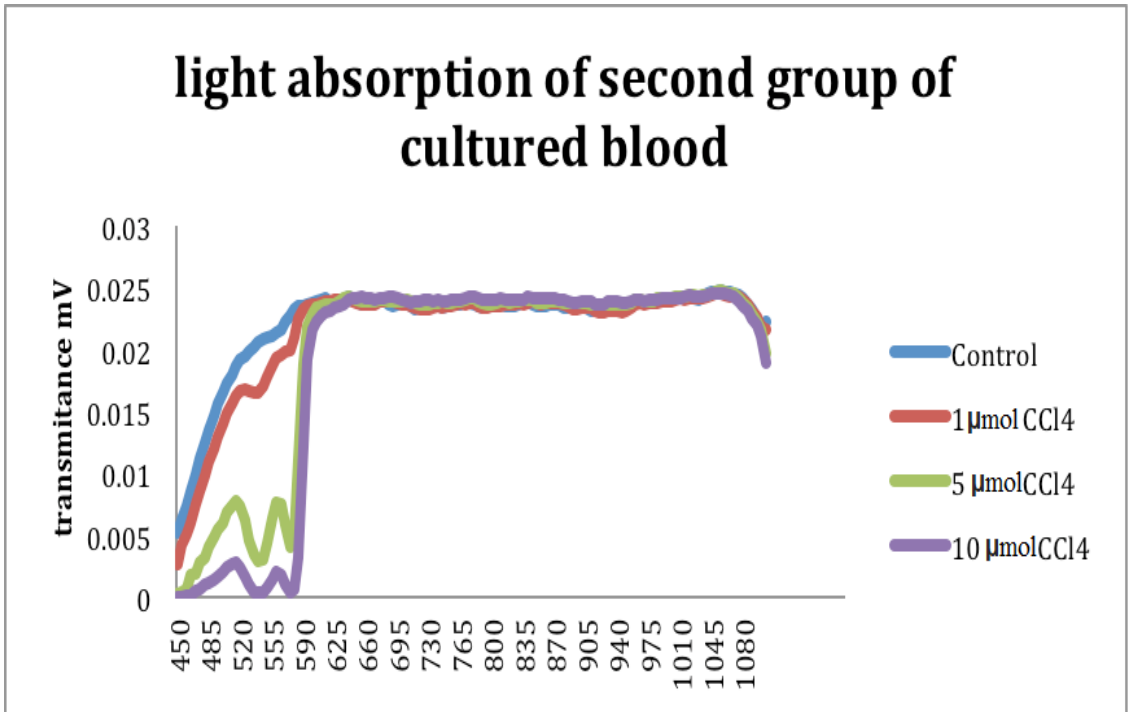

Figure 4.2 Optical response of the second group of effects of different concentrations of CCl_4 in cultured blood cells to the light spectrum from 450 nm to 1100 nm.

The NIR region at all concentrations was similar but the difference was observed at VIS region of light. The transmittance were higher at (1 µmol) CCl_4 comparing to (5 µmol) CCl_4 and (10 µmol) CCl_4, also at the same region the transmittance of (5 µmol) CCl_4 were higher than (10 µmol) CCl_4.

Third group were included (1 µmol, 3 µmol and 5 µmol) of CCl_4 after exposure to culture blood we observed increasing in the average of light absorption of (1 µmol and 3 µmol) CCl_4, but there were decreasing at (5 µmol) CCl_4 compared to control. Increasing (1 µmol) CCl_4 were significantly different ($p<0.05$) while at (3 µmol) were not significant ($p>0.05$). In case of (5 µmol) CCl_4 decreasing were strongly significant ($p<0.05$) as shown in (table 3.2).

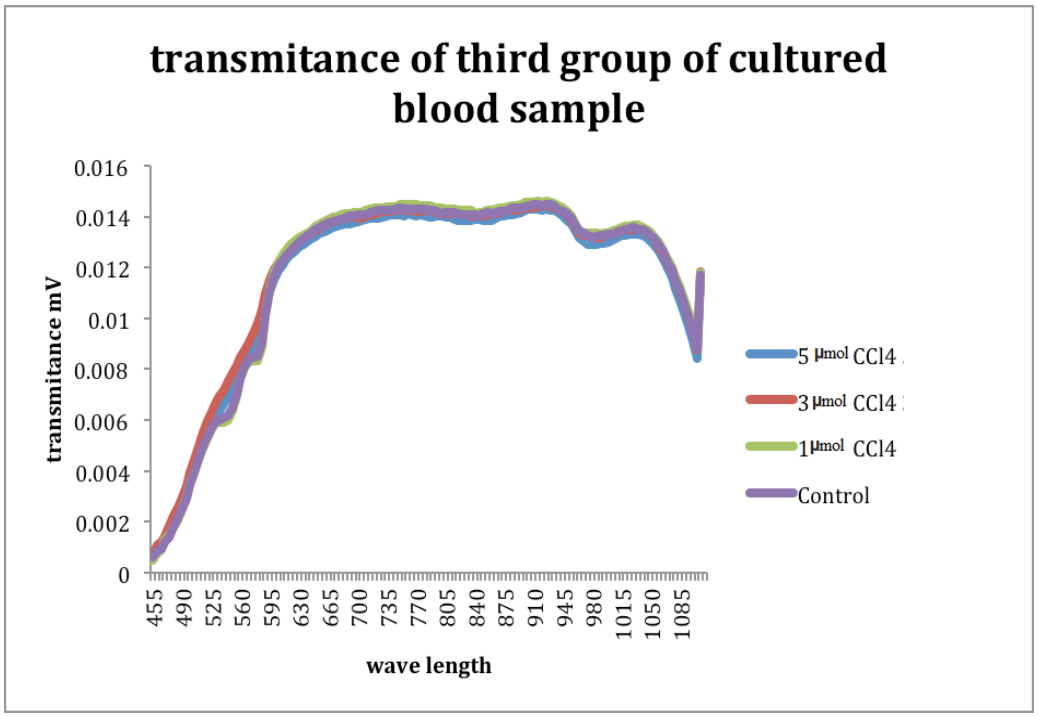

Figure 4.3 Optical response of the third group of effects of different concentrations of CCl_4 on cultured blood cells to the light spectrum from 450 nm to 1100 nm.

In the third group at both region NIR and VIS transmittance were similar at all concentrations. (3 µmol) CCl_4 were higher at NIR comparing to (1 µmol) and (5 µmol) CCl_4 and (5 µmol CCl_4 transmittance were higher than (1 µmol) CCl_4.

In the fourth group were decreased at both concentrations (1 µmol and 5 µmol) CCl_4, the average light absorptions were significantly different at (1 µmol) CCl4 ($P<0.05$), while it was not significant at (5 µmol) CCl_4 ($p>0.05$) as shown in (table 3.2).

In the figure 3.4 observes with the increasing of the concentration of CCl_4 the light transmission will decrease correspond at VIS region, but at NIR region the carves are similar and didn't observes apparently.

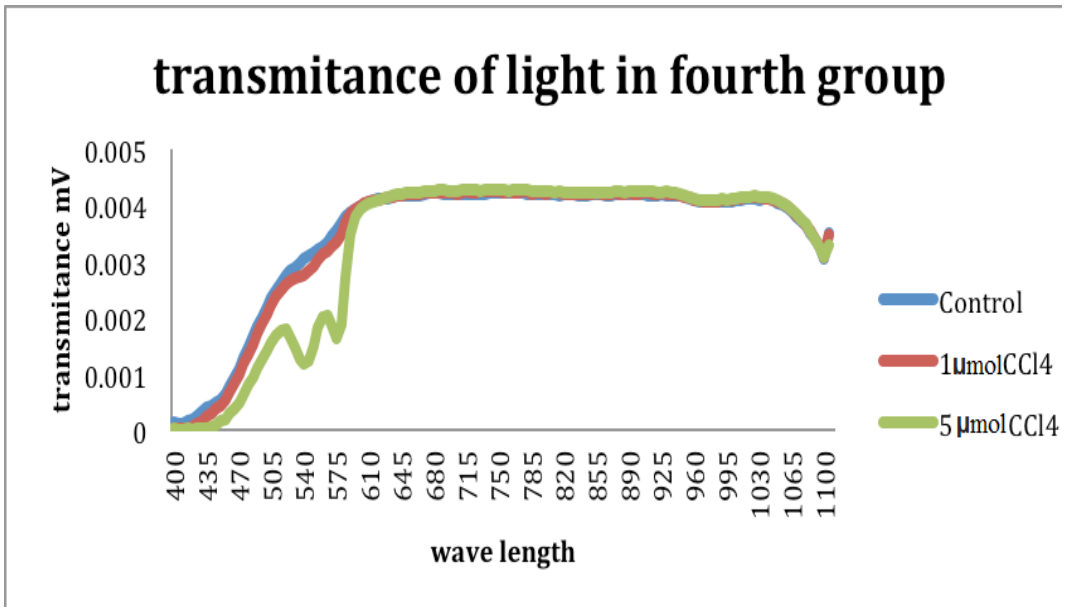

Figure 4.4 Optical response of the fourth group of effects of different concentrations of CCl_4 in cultured blood cells to the light spectrum from 450 nm to 1100 nm.

The wavelength used in this study was ranged (400-1100). This range of wave length includes two regions (VIS and NIR).

VIS includes 400-750 nm in this study we used (NIR ranged from 750-1100nm). All groups with their concentrations shows there were not significant different at NIR region ($p>0.05$).

The difference in transmission was at VIS region as shows in figure (3.1, 3.2 and 3.4) except third group figure (3.3) of blood.

Table 4.3 Mean and t-test for each concentration of CCl_4 added to the groups of cultured blood at VIS region the wavelengths starting from 400 nm ending with 750 nm.

		Concentration CCl_4 in µmol				
Groups of blood	Statistical tests	Control	1 µmol	3 µmol	5 µmol	10 µmol
First group	Mean T test	0,010119508	0,013140752 4,3623E-17*	-	0,012051166 0,000138207	0,011544461 0,814414097
Second group	Mean T test	0,01437469	0,013115657 2,7403E-09*	-	0,012057072 1,38466E-08	0,012015586 5,7803E-09*
Third group	Mean T test	0,007454669	0,007405668 9,646E-05**	0,007407577 1,60754E-08	0,007348808 0,647853625	-
Fourth group	Mean T test	0,004348032	0,004295262 9,084E-10**	-	0,004266548 9,395E-09**	-

As shown in table above there are elevation in the absorption in light transmission in first group, this increasing were significant at 1 **µ**mol and 10 **µ**mol CCl_4 while at 5 **µ**mol were not significant at a level of significant ($P>0.05$). Second group of blood samples were shown that there were significant decreasing of the light absorption at 1 **µ**mol and 10 **µ**mol but increment were not significant at 5 **µ**mol. There are decreasing in the average of the light transmission in third group but the different were only at 1 **µ**mol at a level of significant ($p<0.05$). Fourth group were shows also decreasing in the average of the absorption in both concentrations 1 **µ**mol and 5 **µ**mol both were significant at a level of significantly ($P<0.05$).

In the table 3.3 were observed in all groups the changes were happen significantly in both 1 **µ**mol and 10 **µ**mol CCl_4 concentrations but at 3 **µ**mol and 5 **µ**mol were not significant except in fourth group. This may because at 1 **µ**mol CCl_4, blood cells were in the active state and generate greater antioxidant agent to neutralize the effect of CCl_4 [42]. At both 3 **µ**mol and 5 **µ**mol there were not significant this may also because the blood cells are get to inactive state because of cell death or nucleus dysfunctions [43], at (10 **µ**mol) CCl_4 the changes were significant may due to disruption of the blood cells because of high concentration of CCl_4 correspond leaded to change in the concentration of blood fluid or may leads to generate more amount of chemokines so as to neutralize the high concentration of CCl_4 [44].

Table 4.4 Mean and t-test for total of same concentrations of CCl_4 at all groups of cultured blood at each wavelength starting from 450 nm ending with 1100 nm.

	CCl_4 Concentrations								
	Control	**1 µmol CCl_4**		**3 µmol CCl_4**		**5 µmol CCl_4**		**10 µmol CCl_4**	
Wave length	**Avg.**	**Avg.**	**T test**	**Avg.**	**T test**	**Avg.**	**T test**	**Ave.**	**T test**
450	0,01242	0,00678	0,07908	0,00112	0,20236	0,00205	0,16140	0,00008	0,13279
455	0,01424	0,00930	0,16328	0,00162	0,22327	0,00292	0,15722	0,00013	0,12510
460	0,01607	0,01120	0,16914	0,00186	0,22088	0,00391	0,16385	0,00024	0,12818
465	0,01854	0,01386	0,18014	0,00214	0,21562	0,00593	0,15767	0,00049	0,12742
470	0,02087	0,01682	0,18665	0,00279	0,23534	0,00738	0,17427	0,00064	0,13169
475	0,02342	0,01941	0,19739	0,00339	0,24155	0,00947	0,17020	0,00121	0,13789
480	0,02553	0,02235	0,27573	0,00384	0,24492	0,01149	0,18635	0,00138	0,13686
485	0,02749	0,02492	0,37228	0,00450	0,25529	0,01372	0,19702	0,00194	0,14177
490	0,02942	0,02759	0,55706	0,00517	0,26788	0,01579	0,20880	0,00206	0,13952
495	0,03142	0,03020	0,72655	0,00594	0,28379	0,01774	0,22196	0,00260	0,14104
500	0,03325	0,03277	0,88816	0,00669	0,29787	0,01985	0,24175	0,00315	0,14717
505	0,03493	0,03499	0,99318	0,00745	0,31402	0,02214	0,25694	0,00367	0,14976
510	0,03625	0,03712	0,87310	0,00825	0,34053	0,02406	0,28138	0,00416	0,15206
515	0,03757	0,03873	0,84158	0,00895	0,36200	0,02526	0,29551	0,00422	0,14809

520	0,03861	0,03982	0,84188	0,00954	0,37887	0,02542	0,29420	0,00368	0,12853
525	0,03947	0,04028	0,90366	0,01001	0,39194	0,02376	0,26765	0,00259	0,10366
530	0,04017	0,04007	0,96426	0,01044	0,41248	0,02077	0,23934	0,00132	0,08502
535	0,04071	0,03972	0,83991	0,01079	0,42911	0,01848	0,22112	0,00070	0,07675
540	0,04130	0,03993	0,79555	0,01127	0,45364	0,01759	0,21802	0,00073	0,07695
545	0,04190	0,04118	0,88307	0,01177	0,47558	0,01860	0,22670	0,00053	0,07337
550	0,04260	0,04342	0,92110	0,01227	0,49058	0,02213	0,25078	0,00111	0,07560
555	0,04343	0,04575	0,73635	0,01272	0,49729	0,02677	0,28916	0,00181	0,07877
560	0,04435	0,04735	0,66769	0,01317	0,50618	0,02986	0,32127	0,00284	0,08737
565	0,04503	0,04825	0,65705	0,01361	0,52662	0,03033	0,33266	0,00265	0,08313
570	0,04566	0,04863	0,70104	0,01413	0,56008	0,02751	0,29782	0,00152	0,07133
575	0,04617	0,04900	0,73422	0,01473	0,60234	0,02414	0,26908	0,00074	0,06502
580	0,04720	0,05156	0,62958	0,01556	0,64393	0,02704	0,29758	0,00103	0,06573
585	0,04867	0,05567	0,47534	0,01649	0,67887	0,03949	0,51729	0,00436	0,09609
590	0,04983	0,05832	0,40940	0,01732	0,71240	0,05195	0,86363	0,01754	0,75812
595	0,05059	0,05953	0,39251	0,01788	0,74187	0,05705	0,53743	0,02842	0,26017
600	0,05106	0,06003	0,38950	0,01831	0,77126	0,05878	0,46030	0,03184	0,15009
605	0,05128	0,06038	0,38593	0,01866	0,80179	0,05989	0,43757	0,03316	0,12602
610	0,05147	0,06061	0,38913	0,01900	0,82728	0,06034	0,43243	0,03395	0,11486
615	0,05174	0,06105	0,38397	0,01923	0,84184	0,06059	0,42601	0,03423	0,11148
620	0,05175	0,06139	0,36864	0,01942	0,86076	0,06049	0,41464	0,03447	0,10387
625	0,05193	0,06170	0,36185	0,01963	0,87621	0,06074	0,40119	0,03476	0,09892
630	0,05228	0,06181	0,37185	0,01982	0,88608	0,06131	0,39967	0,03502	0,09913
635	0,05253	0,06168	0,38736	0,01993	0,88745	0,06188	0,39777	0,03523	0,09896
640	0,05263	0,06163	0,39622	0,02005	0,89782	0,06229	0,39631	0,03549	0,09608
645	0,05253	0,06192	0,38832	0,02021	0,92104	0,06214	0,39531	0,03572	0,09071
650	0,05246	0,06195	0,38211	0,02037	0,94036	0,06192	0,38818	0,03570	0,08871
655	0,05240	0,06178	0,37547	0,02049	0,95944	0,06162	0,38032	0,03557	0,08621
660	0,05246	0,06169	0,37763	0,02062	0,97350	0,06162	0,38700	0,03538	0,08850
665	0,05233	0,06178	0,37868	0,02069	0,98918	0,06192	0,38238	0,03532	0,08813
670	0,05264	0,06179	0,38716	0,02077	0,98370	0,06211	0,39634	0,03517	0,09395
675	0,05293	0,06185	0,38873	0,02087	0,98176	0,06223	0,40185	0,03531	0,09578
680	0,05283	0,06221	0,38315	0,02092	0,99097	0,06208	0,39473	0,03551	0,09268
685	0,05262	0,06242	0,36592	0,02096	0,99221	0,06176	0,38512	0,03564	0,08669
690	0,05271	0,06218	0,36252	0,02100	0,99421	0,06183	0,38511	0,03548	0,08778
695	0,05277	0,06191	0,37768	0,02101	0,99544	0,06211	0,39032	0,03538	0,09054
700	0,05254	0,06177	0,38690	0,02097	0,99054	0,06223	0,38759	0,03523	0,09275
705	0,05279	0,06178	0,39275	0,02111	0,98645	0,06220	0,38703	0,03511	0,09640
710	0,05266	0,06179	0,37531	0,02118	0,97171	0,06203	0,37253	0,03506	0,09376
715	0,05236	0,06170	0,37494	0,02118	0,95442	0,06161	0,37868	0,03513	0,08920
720	0,05227	0,06148	0,38376	0,02126	0,94246	0,06154	0,38439	0,03519	0,08812
725	0,05246	0,06137	0,39521	0,02135	0,94039	0,06153	0,39241	0,03498	0,09220
730	0,05252	0,06145	0,39710	0,02131	0,94738	0,06169	0,39249	0,03501	0,09314
735	0,05254	0,06163	0,39726	0,02133	0,94574	0,06182	0,39183	0,03497	0,09447
740	0,05269	0,06196	0,38788	0,02144	0,94052	0,06197	0,38450	0,03502	0,09376
745	0,05263	0,06202	0,37383	0,02145	0,93291	0,06210	0,37376	0,03502	0,09138
750	0,05266	0,06187	0,37669	0,02140	0,94153	0,06194	0,38530	0,03502	0,09256
755	0,05259	0,06177	0,38058	0,02140	0,94102	0,06222	0,38414	0,03496	0,09418
760	0,05247	0,06191	0,37186	0,02141	0,93219	0,06222	0,38288	0,03501	0,09262
765	0,05257	0,06211	0,37193	0,02134	0,94506	0,06225	0,38518	0,03511	0,09277
770	0,05297	0,06219	0,38773	0,02133	0,96468	0,06250	0,38512	0,03524	0,09493
775	0,05303	0,06231	0,37477	0,02138	0,96111	0,06258	0,37554	0,03539	0,09153
780	0,05295	0,06222	0,36684	0,02139	0,95536	0,06240	0,37321	0,03550	0,08933
785	0,05260	0,06183	0,37517	0,02134	0,94716	0,06208	0,37916	0,03532	0,08883
790	0,05230	0,06161	0,37816	0,02127	0,93914	0,06195	0,37753	0,03527	0,08755
795	0,05235	0,06165	0,38539	0,02119	0,95384	0,06181	0,38395	0,03533	0,08878
800	0,05269	0,06177	0,39618	0,02118	0,97168	0,06182	0,39831	0,03542	0,09164
805	0,05295	0,06180	0,39737	0,02117	0,98147	0,06198	0,39626	0,03552	0,09194
810	0,05300	0,06181	0,38910	0,02124	0,97670	0,06197	0,39136	0,03545	0,09177
815	0,05274	0,06184	0,37484	0,02120	0,97042	0,06194	0,38265	0,03535	0,09006
820	0,05251	0,06195	0,37277	0,02115	0,96576	0,06177	0,38628	0,03520	0,09031
825	0,05232	0,06207	0,36981	0,02111	0,96253	0,06185	0,37733	0,03522	0,08870
830	0,05263	0,06219	0,37614	0,02105	0,98261	0,06210	0,38462	0,03531	0,09131
835	0,05300	0,06202	0,39644	0,02107	0,99626	0,06229	0,39061	0,03533	0,09576

840	0,05311	0,06205	0,38880	0,02106	0,99446	0,06224	0,39227	0,03545	0,09387
845	0,05282	0,06207	0,36802	0,02105	0,99216	0,06207	0,38135	0,03538	0,09060
850	0,05248	0,06188	0,36636	0,02114	0,96512	0,06179	0,38474	0,03534	0,08728
855	0,05226	0,06181	0,36791	0,02120	0,94661	0,06180	0,38233	0,03527	0,08667
860	0,05235	0,06214	0,36853	0,02124	0,94899	0,06192	0,38381	0,03524	0,08858
865	0,05259	0,06238	0,36897	0,02130	0,95108	0,06203	0,38131	0,03532	0,08962
870	0,05278	0,06227	0,37392	0,02134	0,95225	0,06215	0,38471	0,03533	0,09145
875	0,05282	0,06203	0,37123	0,02142	0,94482	0,06216	0,38622	0,03525	0,09271
880	0,05243	0,06168	0,36836	0,02144	0,92630	0,06197	0,38210	0,03504	0,09063
885	0,05209	0,06138	0,37377	0,02143	0,91104	0,06189	0,37509	0,03493	0,08950
890	0,05207	0,06130	0,38293	0,02150	0,90144	0,06181	0,37645	0,03489	0,09133
895	0,05227	0,06136	0,38543	0,02152	0,90597	0,06192	0,37484	0,03495	0,09245
900	0,05247	0,06150	0,37896	0,02153	0,91459	0,06201	0,37255	0,03507	0,09134
905	0,05243	0,06148	0,37488	0,02158	0,90696	0,06197	0,37303	0,03511	0,08942
910	0,05203	0,06129	0,36323	0,02161	0,88360	0,06184	0,36352	0,03505	0,08595
915	0,05185	0,06096	0,37621	0,02164	0,87064	0,06174	0,36900	0,03490	0,08895
920	0,05195	0,06108	0,39336	0,02165	0,87386	0,06191	0,37683	0,03491	0,09068
925	0,05212	0,06109	0,40639	0,02164	0,88551	0,06182	0,38604	0,03497	0,09171
930	0,05218	0,06127	0,40335	0,02157	0,89880	0,06187	0,38085	0,03512	0,09013
935	0,05203	0,06144	0,39364	0,02145	0,90484	0,06174	0,37284	0,03517	0,08756
940	0,05188	0,06132	0,38356	0,02137	0,90723	0,06151	0,37209	0,03513	0,08564
945	0,05175	0,06110	0,39310	0,02116	0,92953	0,06134	0,38203	0,03509	0,08625
950	0,05173	0,06106	0,40419	0,02085	0,96819	0,06128	0,39241	0,03510	0,08910
955	0,05166	0,06114	0,40284	0,02042	0,98377	0,06125	0,39584	0,03524	0,08884
960	0,05187	0,06148	0,40548	0,02006	0,93415	0,06144	0,39998	0,03528	0,09230
965	0,05168	0,06149	0,39706	0,01992	0,92809	0,06136	0,39846	0,03542	0,08912
970	0,05151	0,06142	0,39180	0,01990	0,93294	0,06111	0,40156	0,03531	0,08923
975	0,05144	0,06141	0,38246	0,01988	0,93212	0,06114	0,39619	0,03547	0,08693
980	0,05156	0,06143	0,38329	0,01978	0,91331	0,06115	0,40029	0,03543	0,08818
985	0,05171	0,06150	0,38935	0,01979	0,90934	0,06146	0,39621	0,03558	0,08777
990	0,05190	0,06165	0,38763	0,01993	0,91774	0,06157	0,39785	0,03557	0,08882
995	0,05203	0,06172	0,38925	0,02000	0,91995	0,06175	0,39785	0,03569	0,08860
1000	0,05199	0,06176	0,39005	0,02002	0,92295	0,06180	0,39334	0,03563	0,08932
1005	0,05225	0,06194	0,38513	0,02010	0,92273	0,06207	0,39315	0,03576	0,08988
1010	0,05254	0,03885	0,39895	0,02016	0,91898	0,06223	0,38957	0,03588	0,09061
1015	0,05261	0,06218	0,38839	0,02024	0,92298	0,06217	0,39222	0,03593	0,08998
1020	0,05255	0,06237	0,37711	0,02030	0,92952	0,06240	0,38649	0,03602	0,08737
1025	0,05259	0,06236	0,37447	0,02024	0,92295	0,06231	0,38500	0,03599	0,08834
1030	0,05260	0,06230	0,37163	0,02031	0,93018	0,06214	0,38486	0,03590	0,08896
1035	0,05286	0,06235	0,38773	0,02026	0,91258	0,06247	0,39336	0,03603	0,09078
1040	0,05314	0,06255	0,39336	0,02003	0,87796	0,06254	0,40308	0,03612	0,09339
1045	0,05324	0,06270	0,38898	0,01990	0,85876	0,06262	0,39902	0,03635	0,09243
1050	0,05337	0,06268	0,39085	0,01970	0,83281	0,06256	0,40528	0,03639	0,09341
1055	0,05333	0,06249	0,39764	0,01921	0,78411	0,06250	0,39943	0,03636	0,09568
1060	0,05301	0,06202	0,39832	0,01886	0,75841	0,06196	0,40446	0,03632	0,09399
1065	0,05255	0,06156	0,40044	0,01830	0,72056	0,06147	0,41122	0,03627	0,09285
1070	0,05229	0,06127	0,39052	0,01780	0,68547	0,06105	0,41153	0,03608	0,09526
1075	0,05171	0,06052	0,38711	0,01726	0,65575	0,06018	0,42115	0,03570	0,09742
1080	0,05097	0,05962	0,39081	0,01659	0,61983	0,05886	0,43647	0,03511	0,10026
1085	0,04982	0,05841	0,37302	0,01596	0,59952	0,05768	0,42656	0,03444	0,10027
1090	0,04871	0,05710	0,37912	0,01513	0,56025	0,05616	0,43612	0,03371	0,10270
1095	0,04717	0,05535	0,35731	0,01419	0,52273	0,05418	0,43006	0,03282	0,10182
1100	0,04578	0,05305	0,38077	0,01322	0,48096	0,05214	0,44807	0,03160	0,10921

To insure and find a significant difference at exact wavelength the average of all concentration levels with controls is compared at each wavelength were used starting from 450 to 1100 nm as shows in table (3.4).

From mentioned table there were no significant different between blood sera at all CCl_4 concentrations with control at all wave length.

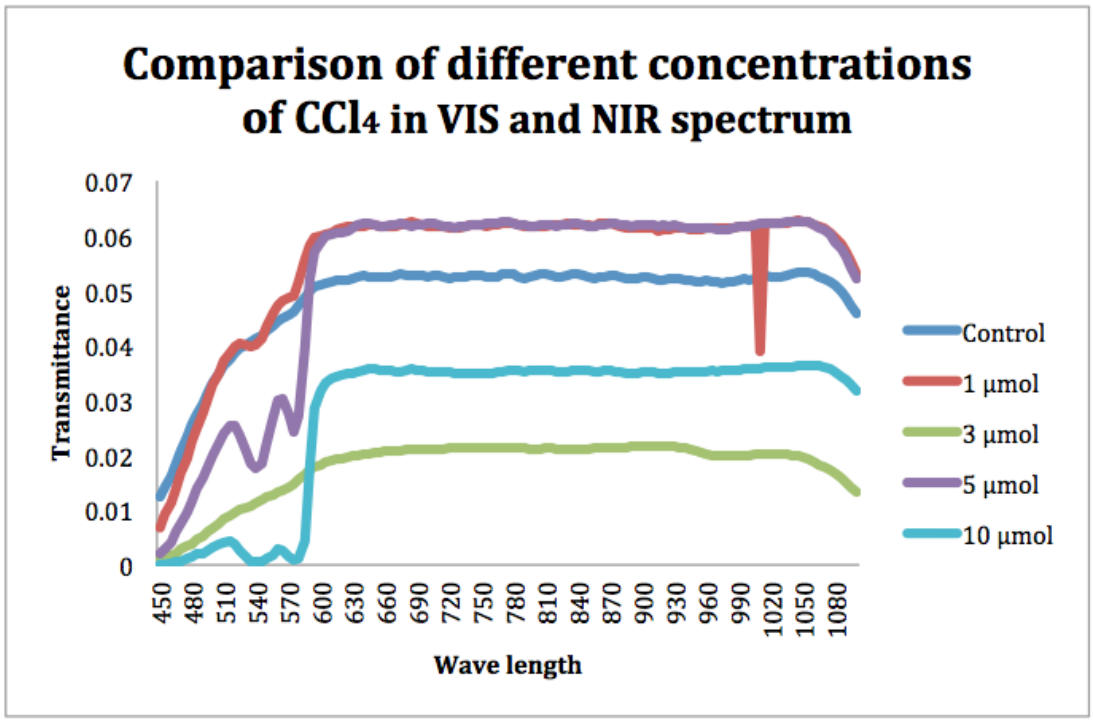

Figure 4.5 Optical response of the all group of effects of different concentrations of CCl_4 in cultured blood cells to the light spectrum from 450 nm to 1100 nm.

In figure (3.5) the transmittance of light of control were starting higher than all CCl_4 concentrations at 450 nm the transmittance were increased with the increasing of wavelength of light. Again control samples are recorded higher transmittance level at visible region. At NIR region both 1 µmol and 5 µmol CCl_4 the transmittance levels were higher than controls 3 µmol and 10 µmol transmittance levels were. Lower according to Figure 3.5 than control a lowest transmittance level were recorded at 3 µmol at NIR region.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

In biological and biomedical studies there are many spectroscopic tools suitable for investigation and indication of specific or special compounds in samples. Not all of them are useful for all kinds of studied samples. All compounds can absorb beams of light in UV, visible, infrared and NIR regions, and also there are other techniques used for measurements such as fluorescence spectroscopy. There is infrared spectroscopy which is widely used in many fields of chemistry including biochemistry and investigative process.

In recent and last decade there are new aspects using spectroscopes which expanded in use to biomedical area. NIR become wide use and more applicable for different fields, however mostly UV and visible regions are used together for biological studies [45]. This study provides measurements that overviewed the absorptions of light in visible and NIR region which compared to MDA standard method. The changes of absorption and reflection of light that is at the range of VIS to NIR, in blood samples exposed to oxidative stress were the field of interests. At visible and NIR ranges, the opportunities offer for detection of induced oxidative stress in cultured blood by CCl_4. Serum absorptions were measured corresponding to concentrations of CCl_4 at all groups. Combination of visible–NIR region together for detect the absorption of light in serum were studied, change in absorption mostly depends on rate of cells broken caused by CCl_4, or changes density of serum because of production of extra antioxidant by lymphocytes. At all groups visible region were showed difference according to CCl_4.

concentrations in serum, but NIR were showed similar. There were no any significant changes at any wavelength at both regions visible and NIR ranging from 450 -1100 nm. This study gave a key to works on the other region of light using same device such as UV and IR ranges of light. Using IR region may gives an opportunity to investigate to apply zolix device and also may find an exact wavelength for detection of such kind of biochemical changes in sera because at IR region of light mostly no require for adding reagents or chemicals to react with specific elements in sera.

It is recommended that, in many biological experiments have used lights or lasers beam for detection of experimental material, different dyes and reactive agents. So that results will be more specific and sensitive. However the type and working principle of used devices are important, besides sensitivity of detector to give accurate results. Indeed zolix is one of the sensitive device and have good enough detectors that provides and accurate results. Using specific dyes or reagent for the present study may gives better and more sensitive results. So it is much better to use sufficient reagent as a detector for measurement experimental cases and biomedical reactions will be more dependable and trustworthy.

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