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PHYSICS

FOURIER TRANSFORM INFRARED SPECTROSCOPY AND FLUORESCENCE EMISSION SPECTROSCOPIC INVESTIGATIONS ON RAT TISSUE

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Abstract

Fluorescence spectroscopy is an emerging technology in the study of biological samples like tissues. When a tissue sample is illuminated with specific wavelength of UV or visible light, it results in the excitation of fluorescent biological molecules known as fluorophores. They absorb the energy and emit it in the form of fluorescent light of longer wavelengths. This property can be effectively employed in the diagnosis of tissues under various conditions. FTIR spectroscopy offers an elegant and easy approach to clinical analysis. The use of FTIR spectroscopy for biomedical applications has increased tremendously in recent years. FTIR spectrum exhibits characteristic absorption frequencies depending on the difference in their functional groups present in the sample. These techniques can be employed to identify the analytes present in the sample. FTIR spectroscopy can be effectively employed in the tissue analysis. The FTIR spectrum of the tissue is used as a fingerprint in the identification of normal tissue and that affected with the diseased sample. In the present work, both infrared and fluorescence spectroscopy have been employed in the analysis of tissue of different organs of rat. The experimental studies have been carried out on different types of tissue samples such normal, tumor induced and tumor treated cases. The study shows that the spectral profiles are different when the tissue of a particular organ is affected with tumor. But on treatment, the approach of the spectra to the normal condition clearly establishes the effectiveness of the treatment.

Introduction

The exploration of new methods and techniques for the diagnosis of tumors has been very rapid in the recent years. Optical spectroscopy becomes the basis for intense research towards the development of novel, non invasive technique for tissue diagnosis. The measurement of fluorescence from tissue has become a clinical diagnostic tool, especially to assess tissue malignancy or tissue function. Changes in the spectroscopic properties of pathological specimens including diverse tumors originating in many organs such as skin, gastrointestinal tract and oral mucosa have stimulated a great deal of interest for its potential application for the detection and treatment of tumor[1]. Several studies have been performed to define the potential of autofluorescence for cancer diagnosis. Alfano et al[2] was one of the first to observe different spectral profiles of endogenous fluorophores in normal tissues of rat (kidney, prostrate) and mouse (bladder) and cancerous tissues. Karthikeyan et al[3] and Masilamani et al[4] have shown that there is a relationship between porphyrin (with fluorescence at 630 nm) and cancer cell proliferation in an animal tumor model. Wenchong et al[5] wrote about a study of cancerous blood and tissues using porphyrin as a tumour marker. Infrared spectroscopy is a powerful method for the study of molecular structure and intermolecular interaction in biological tissues and cells. Feride et al[6] studied the effect of sreptozotocin (STZ) induced diabetes on rat liver and heart tissues using FT-IR spectroscopy. Chiriboga et al[7] studied infrared spectra of normal and cancer liver tissues such as glycogen, DNA and RNA. Patrick et al[8] studied the human colon tissues at molecular level from normal epithelium to malignant tumor investigation by pressure tunning FT-IR spectroscopy.

In this work, an attempt has been employed to study and analyse the changes in the molecular level in three different types of rat tissues under different conditions namely normal, tumor affected by inducing carcinogenic drugs and treatment of tumor using fluorescence emission spectroscopy (FES) and Fourier transform infrared (FTIR) spectroscopic techniques.

Theory of fluorescence emission (FES) spectroscopy

Absorption of UV radiation by a molecule excites it from a vibrational level in the electronic ground state to one of the many vibrational levels of the electronic excited state. A molecule in a high vibrational level of the excited state will fall off quickly to the lowest vibrational level of this state by losing energy to other molecules through collision. The molecule will also partition the excess energy to other possible modes of vibration and rotation. Molecules that are excited to

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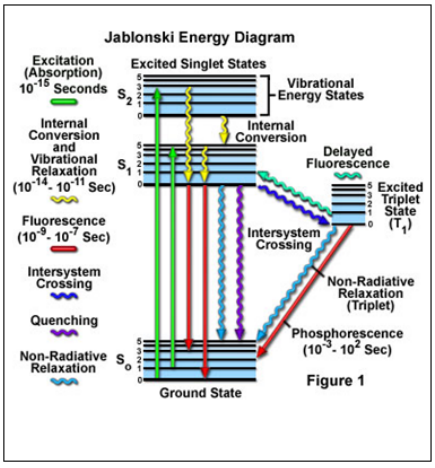
high energy levels can decay to lower levels by emitting radiation. This process is called emission or luminescence.

The phenomenon, *Fluorescence* of substances, had been observed for hundreds of years and was explained by the British scientist Sir George G. Stokes in 1852 that fluorescence can be induced in certain substances by illuminated with ultraviolet light. He formulated Stokes's law, which states that the wavelength of the fluorescent light is always greater than that of the exciting radiation; therefore fluorescence is the phenomenon in which absorption of light of a given wavelength by a fluorescent molecule is followed by the emission of light at longer wavelengths. Thus, fluorescence is the emission that results from the return to the lower orbital of the paired electron. Such transitions are quantum mechanically allowed and the emissive rates are typically near 10^8 sec^{-1}. These high emissive rates result in fluorescence lifetimes near 10 nano seconds.

The lifetime is the average period of time when a fluorescence biomolecule, flourophore remains in the excited state. For atoms excited by a high temperature energy source, this emission is commonly termed as optical emission. For molecules, it is termed fluorescence if the transition occurs between states of the same spin. There are certain factors that control the occurrence of fluorescence. It is seen that whenever the interaction between excited molecules and surrounding is strong, radiation less decay will occur. But if the interaction between them is ineffective at achieving large energy, transfer needs to take it to the lower electronic state. The radiative decay thus dominates and the molecule fluoresces.

The various energy levels involved in the absorption and emission of light by a molecule is classically presented by a Jablonski energy diagram, named in honor of the Polish physicist Professor Alexander-Jablonski.

Figure 1: Jablonski Diagram



A typical Jablonski diagram illustrates the singlet ground (S (0)) state, as well as the first (S (1)) and second (S (2)) excited singlet states as a stack of horizontal lines as shown in Figure 1. The thicker lines represent electronic energy levels; while the thinner lines denote the various vibrational energy states (rotational energy states are ignored). Transitions between the states are illustrated as straight or wavy arrows, depending upon whether the transition is associated with absorption or emission of a photon (straight arrow) or results from a molecular internal conversion or non-radiative relaxation process (wavy arrows). Vertical upward arrows are utilized to indicate the instantaneous nature of excitation processes, while the wavy arrows are reserved for those events that occur on a much longer timescale. Absorption of light occurs very quickly (approximately a femto second, the time necessary for the photon to travel a single wavelength) in discrete amounts termed quanta and corresponds to excitation of the fluorophore from the ground state to an excited state. Likewise, emission of a photon through fluorescence or phosphorescence is also measured in terms of quanta [9-12].

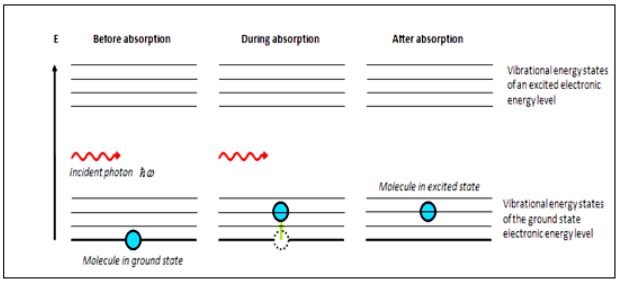
The excess vibrational energy is converted into heat, which is absorbed by neighboring solvent molecules upon colliding with the excited state fluorophore. An excited molecule exists in the lowest excited singlet state (S (1)) for periods on the order of nanoseconds (the longest time period in the fluorescence process by several orders of magnitude) before finally relaxing to the ground state. If relaxation from this long-lived state is accompanied by emission

of a photon, the process is formally known as fluorescence. The closely spaced vibrational energy levels of the ground state, when coupled with normal thermal motion, produce a wide range of photon energies during emission. As a result, fluorescence is normally observed as emission intensity over a band of wavelengths rather than a sharp line.

Theory of Fourier transform infrared (FTIR) Spectroscopy

Infrared (IR) spectroscopy is the powerful and potential analytical tool in the analysis of compounds and molecules. The interaction of molecules with E.M. waves forms the main concept of IR spectroscopy. For a molecule to absorb IR radiations, the vibrations or rotations within a molecule must cause a net change in the dipole moment of the molecule. The alternating electrical field of the radiation interacts with fluctuations in the dipole moment of the molecule. If the frequency of the radiation matches the vibrational frequency of the molecule then the radiation will be absorbed, causing a change in the amplitude of molecular vibration. Molecular vibrations give rise to absorption bands in the IR region of the spectrum. The far IR, lying adjacent to the microwave region has low energy and therefore used for rotational spectroscopy.

Figure 2: Energy levels of a molecule during the absorption of a photon



From Figure 2, it is observed that photons with specific energies will be absorbed by the molecule if this energy is equal to the difference between the energy levels. This is when the frequency of the IR radiation matches the vibrational frequency of the molecule. Before absorption the molecule is in an excited vibrational state but still in the ground state of the electronic energy level. The molecule does not remain in this excited state forever. The energy absorbed by a molecule is rapidly dissipated; it is transformed into kinetic energy as a result of collisions or released again as photon [13, 14].

The development of Fourier Transform Infrared spectroscopy (FTIR) in the early 1970s provided a quantum leap in infrared analytical capabilities. The first FTIR spectrometer was proposed by Fellgett. FTIR techniques have made significant impact with regards to rapid scanning, signal to noise ratio, high sensitivity, high resolution and data processing. With the use of Fourier transformations it is possible to convert a signal in the time domain to the frequency domain. This allows the user to convert intensity versus time spectrum into an intensity-vs.-frequency spectrum.

The Fourier transform is given by,

$$A(r) = \sum X(k) \exp\left(-2\pi \frac{irk}{N}\right)$$

where A (r) and X (k) are the frequency domain and time domain points, respectively, for a spectrum of N points.

FTIR is the most powerful tool for identifying various types of chemical bonds (functional groups). The wavelength of light absorbed is the characteristic of the chemical bond. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. FTIR spectra of pure compounds are generally so unique that they are like a molecular "fingerprint".

Biological tissue

Tissue is a group of cells that have similar structure and that function together as a unit. A non living material, called the intercellular matrix, fills the spaces between the cells. This may be abundant in some tissues and minimal in others. The intercellular matrix contains special substances such as salts and fibers that are unique to a specific tissue and gives

those tissues distinctive characteristics. There are three main types of tissues in the body,

(a) Epithelial tissue
(b) Connective tissue
(c) Muscle tissue

Epithelial tissue is made of closely packed cells arranged in flat sheets. Epithelia form the surface of the skin, line the various cavities and tubes of the body, and cover the internal organs. They are packed tightly together, with no intercellular spaces and only a little amount of intercellular substance. Thus the tissue covers the whole surface of the body. The cells of connective tissue are embedded in a great amount of extra cellular material. This matrix is secreted by the cells. It consists of protein fibers embedded in an amorphous mixture of protein-polysaccharide molecules. Binding connective tissue binds the body parts together. Tendons connect muscle to bone. The matrix is principally collagen, and the fibers are oriented parallel to each other. Ligaments attach one bone to another. They contain collagen and also the protein elastin. Elastin permits the ligaments to be stretched. Fibrous connective tissue serves as a packing and binding material for most of the organs. Collagen, elastin and other proteins are found in the matrix. There are three kinds of muscles found in animals; skeletal muscle made of long fibers whose contraction provides the force of locomotion and other voluntary body movements. Smooth muscle lines the walls of the hollow structure s of the body, such as the intestine, uterus and blood vessels. Its contraction is involuntary and so reduces the size of these hollow organs. The heart is made of cardiac muscle.

Analysis of biological tissues using spectroscopic techniques

Fluorescence emission spectroscopy

The categories of molecules capable of undergoing electronic transitions that ultimately result in fluorescence are known as fluorescent probes, fluorochromes, or simply dyes. Fluorochromes that are conjugated to a larger macromolecule (such as a nucleic acid, lipid, enzyme, or protein) through adsorption or covalent bonds are termed fluorophores. In general, fluorophores are divided into two broad classes, termed intrinsic and extrinsic. Intrinsic fluorophores, such as aromatic amino acids, neurotransmitters, porphyrins, and green fluorescent protein, are those that occur naturally. Extrinsic fluorophores are synthetic dyes or modified biochemicals that are added to a specimen to produce fluorescence with specific spectral properties such as dansyl, fluorescein, rhodamine, and so on. The wavelength and time resolution required of the instruments is determined by the spectral properties of the fluorophores. It is to be noted that the fluorophores are specific in their properties[15 – 19].

The biological molecules which exhibit endogenous fluorescence include amino acids, structural proteins, enzymes and co-enzymes, vitamins, lipids and porphyrin. Their excitation maxima lie in the range 250 nm to 450 nm(in the UV/ Visible spectral range), while their emission maxima lie in the range 280 nm to 700 nm (around UV/Vis/NIR spectral range). The endogenous intrinsic fluorophores that play an important role in transformations that occur with carcinogenesis or any other changes are the amino acids tryptophan and tyrosine, the structural proteins collagen and elastin, the coenzymes NADH and FAD and porphyrins [20].

The following table gives lists of examples of the biological molecules (intrinsic fluorophores) that exhibit endogenous fluorescence, along with their excitation and emission maxima, which provides an important tool in the optical assessment of metabolic status;

Fluorophores	Excitation maximum (nm)	Fluorescence maximum (nm)
Tyrosine	275	300
Tryptophan	280	350
Collagen	300	420
Elastin	300	420
NADH	350	460
Flavins	450	530
Porphyrin	405	635

Action of CCl_4 and Pentoxifylline on rat tissues

Using fluorescence spectroscopy, the present work has been carried out on rat tissues for three different organs namely liver, kidney and heart. All the samples have been excited at 280nm which corresponds to the fluorescence of the aminoacid, tryptophan.

Materials and experimental method

An in vitro study was been performed on rat tissues, under three different categories;

- Normal and healthy rats. (Group A)
- Rats induced with CCl_4 (carcinogen) for 3 days. (Group B)
- Rats induced with CCl_4 (carcinogen) and then with pentoxifylline (anti-tumor drug) for 3 days. (Group C)

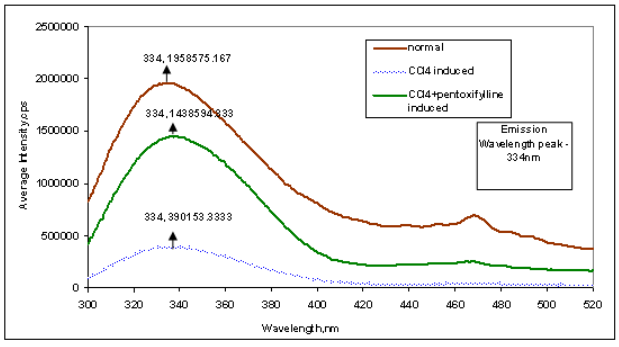
The fluorescent probe taken in this study is the intrinsic (natural) fluorophore tryptophan. The nature of this protein was analyzed separately for liver, kidney and heart tissue samples. Male Wistar rats (Biological name-*Rattus norvegicus)* weighing about 180-220g were fed on a standard laboratory diet and grown. The study groups consisted of normal and healthy rats (Group A), rats induced with 0.2ml/kg wt. of CCl_4 (Group B) in a ratio of 1:1 with olive oil (drug vehicle), and rats treated with a single dosage of CCl_4, then administered with 72mg/kg wt. of pentoxifylline drug (Group C) intraperitoneally, the process being repeated for every 4 hours.

The rats were then sacrificed for the study after obtaining complete ethical clearance at Saveetha University. After the sacrifice, the tissue samples have been collected for conducting an invitro fluorescence emission spectral analysis. The individual samples have been put in separate plastic containers completely filled with normal saline . The fluorescence emission spectra (FES) have been analyzed using an ISA-Spex FluoroMax-2 spectrofluorometer at SAIF, IIT Chennai. Till the spectral analysis, the samples have been stored in deep freezer at a temperature of -17 degree C. On the day of spectral analysis, the tissue sample to be recorded is placed directly in the sample holder in the instrument after the sample is brought to room temperature. The fluorescence emission spectra (FES) were recorded in the region of 400-700nm.

Results and Discussion

The fluorescence emission spectrum of liver tissue samples has been recorded at excitation wavelength of 280nm corresponding to trytophan fluorescence. From the spectra obtained for liver tissue samples, the spectral characteristics of normal tissues differ significantly from those of the CCl_4 induced samples and pentoxifylline treated samples as well. Upon excitation at 280nm, the peak at 334nm is due to the fluorescence of tryptophan amino acid. It is clearly inferred that the concentration and absorption of the amino acid goes very low, than that observed in normal healthy tissue, when CCl_4 is induced. This is indicated by the decrease in the intensity corresponding to the tryptophan emission peak at 334nm. This confirms the denaturation of the tryptophan amino acid due to the influence of the carcinogenic nature of CCl_4, thus causing tumor in the tissue. As pentoxifylline drug is administered to the tissue, it is seen that there is a controlled revival of tryptophan, which is indicated by the rise in the intensity value obtained corresponding to 334nm from the spectrum as shown in Figure 3. Thus, it clearly confirms the carcinogenic nature of CCl_4 which induces tumor by cellular destruction and the effective action of pentoxifylline as anti cancer drug in the liver tissue.

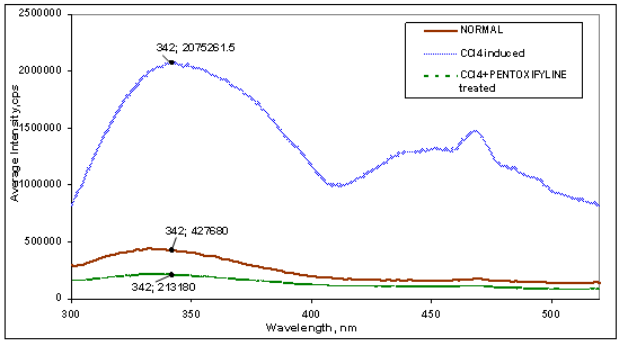
Figure 3: Average Fluorescence spectra of three different liver sample groups-280nm



As a measure to study the activity of CCl_4 on inducing tumor and pentoxifylline on treatment, the experiment was carried out with another tissue of rat, namely kidney. The spectra show a prominent fluorescence peak at 342nm wavelength, but there is a difference in the intensity value between the three study groups as shown in Figure 4. It is observed that when CCl_4 carcinogen is induced to the tissue, there is a sudden increase in the concentration of tryptophan amino acid which is seen from increase in the intensity

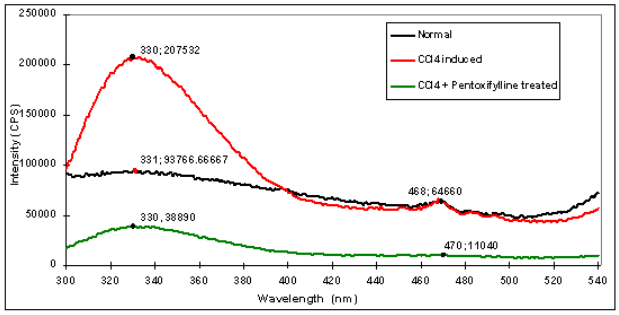
corresponding to the band peak at 342nm when compared with that of the healthy sample group. Similarly, when pentoxifylline is induced as a treatment drug, the abnormal rise in the intensity occurred at the emission peak disappears and attains the actual value of the concentration of the amino acid to a great precision. The sudden rise in the intensity peak may be attributed due to the carcinogenic activity of CCl_4, which leads to the excessive cellular proliferation of the tumor cells. When pentoxifylline drug is administered, the abnormal cell proliferation is curtailed and there is a control reformation of tryptophan amino acid, thereby confirms the tumor curative property of drug.

Figure 4: Average Fluorescence spectra of three different kidney sample groups- 280nm



The experiment is carried out to study the tumor activity in the heart tissue of rat. The spectra show a prominent fluorescence peak at 330nm wavelength, but there is a difference in the intensity value between the three study groups. It is observed that a drastic change in the tryptophan level is produced due to the induced CCl_4 when compared to the normal heart samples. However, the effect of pentoxifylline in treating the tumor is not predominant as seen in the liver and kidney tissues as per Figure 5.

Figure 5: Average Fluorescence spectra of three different heart sample groups-280nm



Thus from the fluorescence emission (FES) analysis, it has been deduced that CCl_4 is a very strong carcinogen and affects all the vital organs in a living organism when induced. Pentoxifylline administration has been, till date shown to produce dose-related hemorrheologic effects, lowering blood viscosity and improving erythrocyte flexibility. Pentoxifylline has been used to inhibit neutrophil adhesion and activation. Tissue oxygen levels are found to increase significantly by therapeutic doses of pentoxifylline in patients with peripheral arterial disease. From this work, Pentoxifylline is observed to be a tumor treatment drug, especially for liver and kidney damage; which is a new developing research study [21].

Action of diethyl notrosamine (DEN) and *Garcinia mangostana* pericarp extract

As an extension of FES studies, the experiment has been carried out to analyse another tumor inducing drug , diethyl nitrosamine (DEN). An attempt has been made to analyse the effectiveness of treatment of tumor by an Ayurvedic drug, *Garcinia mangostana* pericarp extract. A thorough investigation on the effect of CCl_4 on inducing tumor and its treatment by pentoxifylline has been established on three different tissues. Hence the present work on diethyl nitrosamine and *Garcinia mangostana* pericarp extract has been

restricted to only one type of rat tissue namely liver. But the FES study has been carried out for three different fluorophores namely tryptophan, collagen and porphyrin.

Materials and experimental method

Male Wistar rats (Biological name-*Rattus norvegicus)* weighing about 180-220g were fed on a standard laboratory diet. The animals were divided into the following groups;

Group A: Control – 6 rats

Group B: diethyl nitrosamine drug induced (causing tumor) – 6 rats

Group C: diethyl nitrosamine followed by *Garcinia mangostana* pericarp extracts – 6 rats

Group D: *Garcinia mangostana* pericarp extract treated alone – 6 rats

The rats of groups B and C were oral dosed on diethyl nitrosamine which causes hepatocellular carcinoma in the cells at sub-lethal dose. After this the animals of group C were again orally administrated with (mangosteen fruit) pericarp extract each day. The rats of control (group A) enjoyed the laboratory diet alone and tap water adlibitum. Group D rats were administrated with Garcinia mangostana prericarp extract alone and tap water ad-libitum. This was done to ensure if Garcinia mangostana pericarp extract by itself produces any side effects to liver organ. Methanolic fractions of Garcinia mangostana fruit extract are very useful to eliminate the unwanted toxic heavy metal from the animal body through the urine. For that purpose this herbal plant pericarp extract has been administrated on diethyl nitrosamine intoxicated animals.

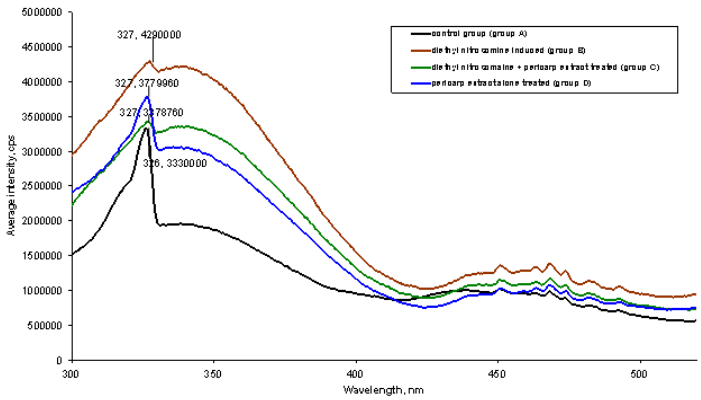
The rats were then sacrificed for the study after obtaining complete ethical clearance at Saveetha University. After the sacrifice, the tissue samples have been collected for conducting an invitro fluorescence emission spectral analysis. The individual samples have been put in separate plastic containers completely filled with normal saline . The fluorescence emission spectra (FES) have been analyzed using an ISA-Spex FluoroMax-2 spectrofluorometer at SAIF, IIT Chennai. During the period of spectral analysis, the samples have been stored in deep freezer at a temperature of -17 degree C. The tissue sample to be recorded is placed directly in the sample holder in the instrument. The fluorescence emission spectra (FES) were recorded in the region of 400-700nm.

Results and Discussion

The liver tissue samples of each of the group were first analyzed by autofluorescence spectroscopic study. The fluorescence emission spectrum of liver tissue samples has been recorded at excitation wavelengths of 280nm, 325nm and 405nm corresponding to tryptophan, collagen and porphyrin fluorescence respectively. The spectra obtained for control tissue samples differ significantly from those of the diethyl nitrosamine induced samples, diethyl nitrosamine + *Garcinia mangostana* pericarp extract treated and *Garcinia mangostana* pericarp extract alone treated treated samples as well as shown in the figure 6.

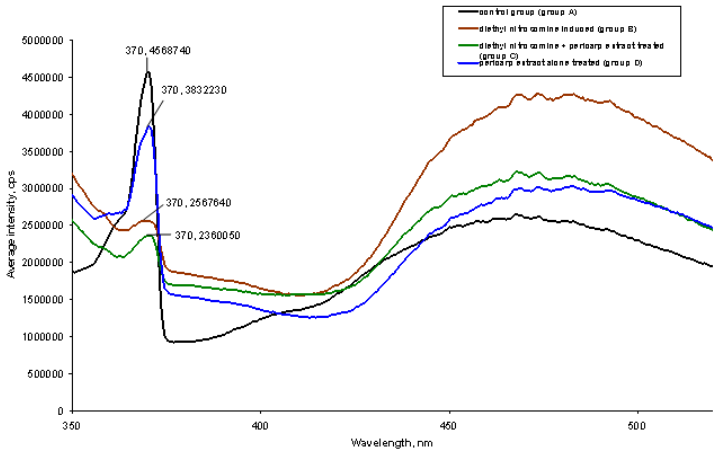
Upon excitation at 280nm, the peak at 327nm is due to the fluorescence of tryptophan aminoacid as shown in Figure 6. It is clearly inferred that the concentration and absorption of the amino acid goes very high, than that observed in normal healthy tissue, when diethyl nitrosamine drug is induced. This is indicated by the proliferation in the intensity corresponding to the tryptophan emission peak at 327nm. This confirms the denaturation of the tryptophan amino acid due to the influence of the carcinogenic nature of diethyl notrosamine, thus causing tumor in the tissue. When *Garcinia mangostana* pericarp extract is administered after inducing diethyl nitrosamine to the tissue (group C), it is seen that there is a controlled revival of tryptophan, which is indicated by the intensity value obtained corresponding to 334nm nearing the intensity obtained for the control group. Similarly the spectrum corresponding to *Garcinia mangostana* pericarp extract alone treated liver tissue samples (group D) indicated there are no side effects by the extract. This is indicated from the intensity at 327nm for group D samples are almost closer to the intensity at 327nm for the control group samples.

Figure 6: Average Fluorescence spectra of four different liver sample groups-280nm



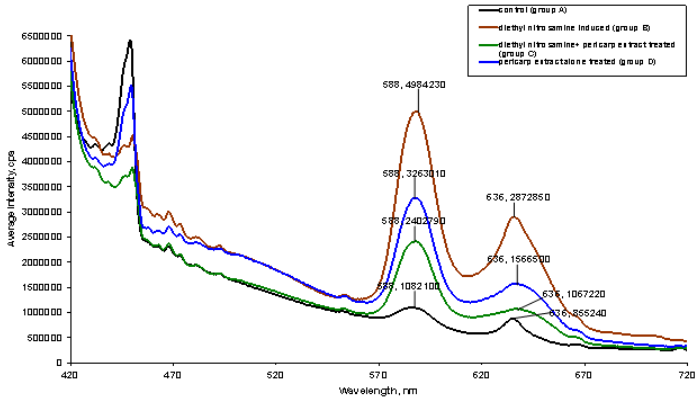
In order to find more about the behaviour of other proteins, fluorescence emission spectra of the samples were studied at 325nm excitation, which occurs due to the fluorescence of the fibrous protein, collagen. The well defined emission band peak at 370nm indicates the concentration and absorption of the fibrous protein, collagen in the tissue as shown in Figure 7. It is observed that there is deterioration of collagen content as diethyl nitrosamine is induced to the tissue, which is marked by the decrease in the fluorescence intensity at 370nm emission wavelength, when compared with the intensity obtained for the control group tissue samples. This confirms the toxic activity of diethyl nitrosamine more effectively. However, it is seen that when *Garcinia mangostana* pericarp extract is administered to the diethyl nitrosamine induced tissue (group C), there is no regeneration of the collagen protein in the tissue. This is observed by the intensity values corresponding to the emission peak at 370nm for group B and group C liver tissue samples as obtained from the spectrum. However it is also observed that the spectrum corresponding to *Garcinia mangostana* pericarp extract alone treated liver tissue samples (group D) indicated there are no side effects by the extract. This is indicated from the intensity at 370nm for group D samples are almost closer to the intensity at 370nm for the control group samples. Thus the *Garcinia mangostana* pericarp extract is observed to be not much significant in the regeneration of the fibrous protein in the liver tissue.

Figure 7: Average Fluorescence spectra of four different liver sample groups-325nm



In order to find about the behaviour of heme content in the tissue, fluorescence emission spectra of the liver tissue samples were studied at 405nm excitation, which occurs due to the fluorescence of porphyrin ring compound. The well defined emission peaks at 588nm and 636nm indicates the presence of heme ring, porphyrin in the tissue samples as shown in Figure 8. It is observed that when diethyl nitrosamine carcinogen is induced to the liver tissue samples (group B), there is a sudden increase in the concentration of porphyrin which is seen from increase in the intensity corresponding to the band peaks at 588nm and 636nm respectively when compared with that of the control group (group A). It is observed that when *Garcinia mangostana* pericarp extract is administered as a treatment drug to the diethyl nitrosamine induced tissue samples (group C), the abnormal rise in the intensity occurred at both the emission peaks disappears and approaches the actual value of the concentration of the amino acid to a great precision, which is shown in Figure. The spectrum peak corresponding to *Garcinia mangostana* pericarp extract alone treated liver tissue samples (group D) indicated there are no side effects by the extract. The sudden rise in the intensity peak may be attributed due to the carcinogenic activity of diethyl nitrosamine, which leads to the excessive cell proliferation of the tumor cells. When *Garcinia mangostana* pericarp extract is administered, the abnormal cell proliferation is curtailed and there is a controlled reformation of porphyrin[22].

Figure 8: Average Fluorescence spectra of four different liver sample groups-405nm



Fourier transform infrared (FTIR) spectroscopy

Spectral bands in vibrational spectra are molecule specific and provide direct information about the biochemical composition. FTIR peaks are relatively narrow and in many cases can be associated with the vibration of a particular chemical bond (or a single functional group) in the molecule. In this technique the molecular-level information allowing investigation of functional groups, bonding types, and molecular conformations is mainly carried out. The vibrational bands are relatively narrow, easy to resolve, and sensitive to molecular structure, conformation, and environment.

Using the sensitivity of FTIR spectroscopy to the biomolecular changes many works has been done in the analysis of tissues. The increased cell proliferation and metabolic activity in the diseased tissue results in changes in the oxidation states of several biochemical species [23-25]. Thus it helps in bringing out the change in the composition of the diseased tissue sample from the healthy tissue sample.

It is seen that tissue proteins, carbohydrates and lipids play a major role as energy provider for animal exposed to stress conditions. A majority of toxic substances initiate biochemical alterations acting at the molecular level by anyone of the following mechanisms:

(i) Inhibition of the enzyme system,

(ii) Altering the level of enzyme and specificity or by

(iii) Altering the permeate properties of body membranes.

Action of diethyl notrosamine (DEN) and Garcinia mangostana pericarp extract

Materials and experimental method

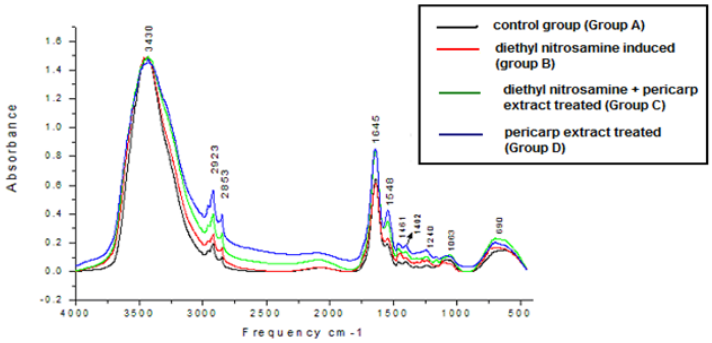
In the present work, FTIR spectral investigation has been carried out on the liver tissues of rat under three different conditions namely normal, tumor induced with diethyl nitrosamine drug, tumor treated with Garcinia mangostana pericarp extract (Groups A to D) as mentioned earlier.

The whole liver tissue samples of each group of rat were isolated. The isolated whole liver tissue samples were lyophilized and made into fine powder. The tissue powder samples and KBr (all solid dry state) were again lyophilized in order to remove most bound water, which might interfere with the prominent group frequencies. 5 mg of liver tissue sample was mixed with 100 mg of dried KBr and subjected to pressure of $5x10^6$ Pa and made into a clear pellet of 13 mm diameter and 1mm thickness. Mid Infrared spectra in the region of 400 – 4000 cm-1 were recorded on PERKIN – ELMER Spectrum One FTIR spectrophotometer, equipped with a KBr beam splitter and an air- cooled DTGS (Deuterated Triglycine Sulfate) detected at SAIF, IIT Chennai. The sampling window was scanned as the background and 32 scans were co added with a spectral resolution of $1cm^{-1}$. The spectrometer was continuously purged with dry Nitrogen. The absorption intensity of the peak was calculated using the base line method.

Results and Discussion

FTIR spectra of normal liver tissues (group A), diethyl nitrosamine induced liver tissues (group B), diethyl nitrosamine followed by *Garcinia mangostana* fruit extract treated liver tissues (group C), and *Garcinia mangostana* fruit extract alone treated tissue samples (group D) are shown in Fig. The relative intensities ($\log I_0 / I$) and tentative assignments of fundamental Infrared absorption frequencies are shown in Table 1.

Figure 9: FTIR Spectra of Liver tissues of rats, (a) control, (b) diethyl nitrosamine induced, (c) diethyl nitrosamine followed by *Garcinia mangostana* pericarp extract, (d) *Garcinia mangostana* pericarp extract alone



The infrared spectra of protein are characterized by a set of absorption regions known as the amide region and the C-H region. The most widely used modes in protein structure studies in the amide region are amide I, amide II and amide III. The amide I band arises principally from the C=O stretching vibration of the peptide group. The amide II band is primarily N-H bending with a contribution from C-N stretching vibrations. The amide III absorption is normally weak and arises primarily from N-H bending and C-N stretching vibrations.

The amide absorptions are considered sensitive to protein conformation; hence an increase or a decrease in the ratio of the intensities of the bands at ~1548 cm^{-1} (amide II) and ~1653 cm^{-1} (amide I) could be attributed to a change in the composition of the whole protein pattern. The bands observed at ~1461 cm^{-1} and ~1396 cm^{-1} are mainly due to asymmetric and symmetric CH_3 bending modes respectively of the methyl groups of protein. The sharp bands observed at ~1653 cm^{-1} and at ~1541 cm^{-1} are assigned to the in plane C=O stretching vibration (amide I) and to the C-N stretching/N-H bending vibration (amide II) of the tissue proteins respectively. The amide I band are primarily associated with the stretching motion of the C=O group. This C=O band is sensitive to the environments of the peptide linkage and also depends on the protein's overall secondary structure.

The medium intensity band observed at ~1235 $cm^{-1}$is that of the PO^{2-}asymmetric stretching modes of the phosphodiester indication of phospholipids and the amide III / CH_2 wagging vibration from the glycine backbone and protein side chain. The band at 1065cm^{-1} has been assigned to the symmetry phosphates; the stretching of glycogen also makes a contribution to the intensity of this band.

The relative intensities (log I_o / I) and tentative assignments of fundamental Infrared absorption frequencies are shown in Table 1.

Table 1: Infrared absorption frequencies (cm^{-1}), relative intensities (log I_o / I) and tentative assignments of fundamental frequencies of Liver tissue samples

Frequency cm^{-1}	Control group	Diethyl nitrosamine induced	Diethyl nitrosamine followed by DEN treatment	DEN treated alone	Vibrational Band Assignments
2923	0.15412	0.37530	0.18100	0.32127	CH_2 asymmetric stretching; lipid, protein
2853	0.31856	0.56593	0.33033	0.48957	CH_2 symmetric stretching; mainly lipids, Proteins
1548	0.29612	0.42271	0.30157	0.37870	C–N stretching/ N–H bending; Amide II
1461	0.55120	0.69656	0.63496	0.72217	CH_3 asymmetric bending; Protein
1402	0.64486	0.79386	0.66109	0.77430	CH_3 symmetric bending; Protein
1240	0.73280	0.88284	0.75316	0.89480	PO_2^- asymmteric sretching Amide III
1063	0.81731	-	0.90315	0.84501	PO_2^- symmeteric sretching (glycogen)

The liver synthesizes a great amount of protein and glycogen, which is needed ostensibly for repair of damaged cell organelle and tissue regeneration. The stressful situations mainly disturb the rate of carbohydrate metabolism through the level of glycogen and protein profile in toxicant exposed animal. Glycogen, a reserve energy source decreased during the inducing of diethyl nitrosamine which is seen in Table 1. A fall in glycogen profile in the liver tissue indicates the possibility of glycogenolysis. The depletion of protein profile was also observed in liver tissue of rats when treated with diethyl nitrosamine.

It was observed in this study that the liver tissues of rats showed a remarkable recovery from the tumor effect of diethyl nitrosamine. When the rats were exposed to diethyl nitrosamine and *Garcinia mangostana* extract treatment, they showed a restoration in the level of biochemical constituent profiles in the liver tissue. The recovery could be attributed to the restoration of regulatory function of protein and glycogen by elimination of tumor causing toxicant[22].

Conclusion

Thus Fluorescence emission spectroscopy (FES) and Fourier Transform Infrared (FTIR) spectroscopic studies can be effectively employed in the qualitative and quantitative analyses of rat tissues.

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