



### RESEARCH ARTICLE

#### FTIR-ATR SPECTRAL AND BIOCHEMICAL VARIATIONS IN DIABETES MELLITUS INDUCED BY ALLOXAN IN WISTAR MALE RAT.

Saira khatheeja<sup>1\*</sup>, Prabhakaran, A.R.<sup>2</sup>, Krishna Mohan, S<sup>3</sup>, Selvanathan, P<sup>4</sup> and Safiullah, A<sup>5</sup>.

1. Asst.Professor in Physics Department of Physics,JBAS College for Women,Chennai 600 018.
2. Assoc.Professor in Physics Department of Physics, Pachaiyappa's College, Chennai-600 030.
3. Vice Principal, Saveetha Medical College and Hospital, Thandalam, Chennai 602.
4. Asst.Professor in Saveetha Dental College and Hospital, Chennai-600 077.
5. Lab Director S.S.Diagnostic Centre,Royapettah,Chennai-600 014.

#### Manuscript Info

##### Manuscript History

Received: 18 October 2016  
Final Accepted: 20 November 2016  
Published: December 2016

##### Key words:-

Diabetes Mellitus, FTIR-ATR Spectroscopy, Alloxan Induction, Wistar Rats

#### Abstract

Diabetes Mellitus (DM) is a destructive disease of carbohydrate, fat and protein metabolism affecting a great amount of world's population. The experiment have been made on the White Wistar rats and recipient rats are injected with alloxan (40 mg/kg body weight) subcutaneously. The experiment have developed DM with Clinical symptoms of haematuria, hyperglycemia etc.. Blood glucose values were  $489 \pm 8.48$  mg/dl recorded to assess the DM in experimental animals induced with alloxan. Current study focus on FTIR spectroscopy together with routine techniques as a diagnostic tool in the discrimination of diabetic samples from healthy ones. A variety of alterations in the spectral parameters, such as frequency and signal intensity/area was observed in diabetic rat compared to the control samples. Based on these spectral variations and routine analysis successful differentiation between diabetic and control groups was obtained in different spectral regions. The results of this current study further revealed the power and sensitivity of FTIR spectroscopy in precise and automated early diagnosis of diabetes. FTIR-ATR spectral variations and quantification of bio molecules among control and experimental animals observed are statistically more significant. The importance of FTIR –ATR Spectroscopy technique in evaluation of qualitative and quantitative nature of biochemical components is discussed.

Copy Right, IJAR, 2016., All rights reserved.

#### Introduction:-

Diabetes Mellitus (DM) is a metabolic disorder which includes a heterogeneous group of damages that have a different etiology, which have common hyperglycemia associated with lipid and protein damages. According to International Diabetic Federation the estimated diabetic prevalence in 2010 has raised to 285 million, representing 6.4 % of the world's adult population, with a prediction that by 2030, the number of people with diabetes will have raised to 438 million. With this alarming concern, India has been declared as the "Diabetic Capital of World".

**Corresponding Author:- Saira khatheeja.**

Address:- Asst.Professor in Physics Department of Physics,JBAS College for Women,Chennai 600 018.

Currently 40.9 million people in India suffering from diabetes alone. It is also estimated that by the year 2030, diabetes likely to be the seventh leading cause of death, accounting 3.3% of the total deaths in the world. DM is characterized by abnormally high blood glucose levels due to decreased secretion or effectiveness in function of insulin. This property of DM causes some metabolic disturbances that lead to chronic, irreversible damage to vital organs and systems (Stapleton, S. R, 2000). DM is classified into two types (Kanazawa, Y 1999, Puavilai, G, 1985) Type I diabetes, also known as insulin-dependent diabetes mellitus (IDDM), is characterized by the autoimmune destruction of pancreatic  $\beta$  cells (Thomas, W.H.K., 2000) Type II diabetes, also known as non-insulin-dependent diabetes mellitus (NIDDM), is a complex disease characterized by target organs, such as liver, muscle, and adipocytes. Complications of DM affect nearly all of the organs and systems in the body and a persistent DM can give rise to severe damage to these organs. It has been reported that diabetes affects the nervous system, especially brain (Brands, A. M et al., 2000, Chu, P.C et al., 1986, Wahba, Z.Z, et al., 1988) the sensory system, especially eye (Cunha-Vaz, J.G et al., 1975, Enea, N.A., 1989) causing diabetic retinopathy (Klein, R 1995), the cardiovascular system (Grossman, E. 1996), stomach, intestine the gastrointestinal and the respiratory system (Thomson, A.B, 1983, Schiller, R and Feldman, M, 1990), gall bladder (Atkinson, M and Hosking, D.J, 1983, Goyal, R. K and Spiro, H.M, 1983) and the reproductive system (Hassan A.A et al., 1993). Liver is the central organ in the body that takes role in the control of metabolism in DM. A number of studies have been primarily focused on the quantitation of clinically relevant biomarkers present in blood, plasma and/or urine (glucose, electrolytes, proteins, lipids, hormones etc., (Ollesch, J et al., 2013, Barman, I 2012) or tissue/organ imaging (Olsztyński Janus S. 2012).

Although the functional and pathological abnormalities seen in diabetes are both clinically and experimentally defined, studies continued to understand the exact molecular mechanism of diabetes (Feride Severcan, 2013). Since DM is affecting all vital organs and it is necessary to rule out the early status of pathological condition by adopting simple method to support the existing techniques for the disease management. Earlier literature shows that Raman and infrared (IR) spectroscopy have been widely tested as powerful tools for medical diagnostics offering a great potential for the real time analysis of large sample number in the clinical setting (Lasch, P and Kneipp, J, 2008, Mitchell, A. L 2014 and Petrich, W. 2001). In recent years, FTIR together with chemometric methods, has had an increasingly important role to play in the field of pathology and diagnosis of disease status. Pathological conditions induces changes in content, structure and function of bio molecules in biological systems and these changes can be rapidly and sensitively monitored by FTIR spectroscopy even at very early stage.

To achieve this, author has chosen an animal model to induce diabetes Mellitus and study the biochemical variations using routine and FTIR-ATR spectroscopic study for control and management of disease. Alloxan has been demonstrated to be non-toxic to the human  $\beta$  cells, even in very high doses, because humans have different glucose uptake mechanisms as compared to rodents (Eizirik, D.L, 1994 and Tyrberg, B, 2001). It has been widely used to induce experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan used (Iranloye B.O, 2011). Moreover, Alloxan is most prominent chemical compound used in diabetogenic research. In research it is used for induction of Type 1 diabetes. Alloxan is a urea derivative which causes selective necrosis of the  $\beta$ - cells of pancreatic islets (Etuk, E.U.N.J, 2010). The chemical name of alloxan is 2,4,5,6 tetra oxypyrimidine; 2, 4, 5, 6- pyrimidinetrone, which is an oxygenated pyrimidine derivative which is present as alloxan hydrate in aqueous solution. So, the current study focused on inducing DM in male wistar rat with alloxan and analyse the bio molecules variations adapting routine and FTIR-ATR spectroscopic method.

### Materials and Methods:-

Male wistar rats were housed in the animal house of Research and Development, Saveetha Medical College and Hospital, Thandalam Chennai, India. All experiments were carried out according to the guidelines for care and use of experimental animals, and are approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Study proposal was approved by the Institutional Animal Ethical Committee. Six male wistar rats per cage were housed in polypropylene cages (32.5 × 21 × 14) cm lined with raw husk which was renewed every 48 hours. The animal house was maintained at an average temperature (24.0  $\pm$  2 C) and 30-70 % RH, with 12 hr. light-dark cycle (lights on from 8.00 a.m. to 8.00 p.m.). Animals received human care and were fed with commercial pellet diet and the animals were acclimatized for one week

before the start of the experiment.

#### **Induction of Diabetes Mellitus:-**

Alloxan was prepared by the oxidation of uric acid by nitric acid and the monohydrate form is simultaneously prepared by oxidation of barbituric acid by chromium trioxide. The drug has been noted to its diabetogenic action and the dose of alloxan required for inducing diabetes depends on the animal species and route of administration ( Federiuk, I.F, 2004 ). Alloxan was obtained from Sigma Chemicals Co., St. Louis, MO, USA. Alloxan was dissolved in normal saline and always prepared freshly for immediate. The male wistar rats were divided into two groups: animals from the first group were made subcutaneous injection of Alloxan (40 mg/kg body weight ) for 0,5, 11 and 12<sup>th</sup> days. and animals in the other group were untreated control . On day 14<sup>th</sup> of the experiment animals were sacrificed by decapitation The blood glucose concentration was measured every day from the day of Alloxan induced and blood samples were collected from the tail vein once a day and checked for hyperglycemic condition by accu check method. Animals with a blood glucose concentration 200 mg/dL were considered to be diabetic ( Tranquilur N.C, et al., 2009 and Saud Alarifi et al., 2012 )

#### **Sample preparations for Biochemical studies:-**

At the end of experiment (14<sup>th</sup> day ) the male wistar rats were fasted overnight . Blood samples of the male wistar rats were withdrawn on from the heart. under mild anaesthesia before killing and collected in plain and EDTA tubes for further analysis . Plasma and serum were separated by centrifugation at 3000 rpm for 15 minutes. The blood serum properly stored for estimation of biochemical parameters including, Glucose, Total cholestreol, triglycerides, LDL, urea, creatinine , uric acid, total protein, SGOT, SGPT e t c . , by CLIA and Total serum T<sub>4</sub> ,T<sub>3</sub> and TSH concentration were determined by ELISA (detection kits provided by Transasia , Zemun, SCG) in a reputed clinical laboratory in Chennai . FTIR-ATR Spectral Analysis the serum samples were properly preserved in ice bags and immediately transported to the wet laboratory.

#### **FTIR-ATR spectral Measurements:-**

The FTIR-ATR spectroscopy is based on the phenomenon known as Total Internal Reflection (TIR) . This radiation strikes the interface between the IRE and the serum sample composed of a lower refractive index. This internal reflectance creates an evanescent wave that extends beyond the surface of the crystal into the serum sample held in contact with the crystal. It can be easier to think of this evanescent wave as a bubble of infrared that sits on the surface of the crystal. This evanescent wave protrudes only a few microns (0.5 $\mu$ -5 $\mu$ ) beyond the crystal surface and into the sample. The depth of penetration of infrared radiation from denser IRE into the test material depends on refractive indices of the materials to be investigated and the wave number of the infrared radiation. As the sample absorbs IR radiation at certain frequencies, the resultant totally reflected radiation (or) evanescent wave will be attenuated (altered) in regions of the infrared spectrum where the sample absorbs energy ( Katon JE and Micron 1996, Baulsir CF and Simler RJ 1996 ). This attenuated IR radiation of evanescent wave is passed back to the IR beam, which then exits the opposite end of the crystal and it is detected by the detector in IR spectrometer. The system generates an infrared spectrum.

FTIR-ATR spectral measurements of serum samples of male wistar rat induced diabetes were carried out at Sophisticated Analytical Instrumentation facility (SAIF-SPU), St. Peter's University, Avadi, and Chennai-600 054, using PerkinElmer Spectrum-Two FTIR Spectrophotometer with attenuated Total Reflectance accessory having highly reliable and single bounce diamond as its Internal Reflectance Element (IRE). Experimental serum samples were analyzed immediately for spectral recordings in the Mid IR region of 4000 - 450 cm<sup>-1</sup>. As water is a good absorbent of infrared radiation, it affects the actual spectral response of the test material and dominated in the FTIR spectrum of serum sample and therefore it was placed on the IRE crystal and water content on the serum sample is removed by air drier. FTIR spectral measurements were carried at room temperature and each measurement was repeated to ensure the reproducibility of the spectra. These spectra were subtracted against the background of air spectrum. After every scan, the crystal is cleaned with isopropyl alcohol or methanol soaked tissue and a background of new reference air was taken to ensure the crystal cleanliness.

#### **Statistical Analysis:-**

All statistical analysis were performed using Statistical Package for Social Science (SPSS, version 17) for Microsoft windows. The data were not normally distributed.

and therefore Non - parametric tests were performed. Descriptive statistics were presented as numbers and percentages. The data were expressed as Mean and SD. A one way analysis of variance ( ANOVA ) /Kruskal-Wallis test with a post hoc Tukey HSD was used. Independent sample student t test / Mann-Whitney test were used to compare continuous variables between two groups. A two sided p value < 0.05 was considered statistically significant.

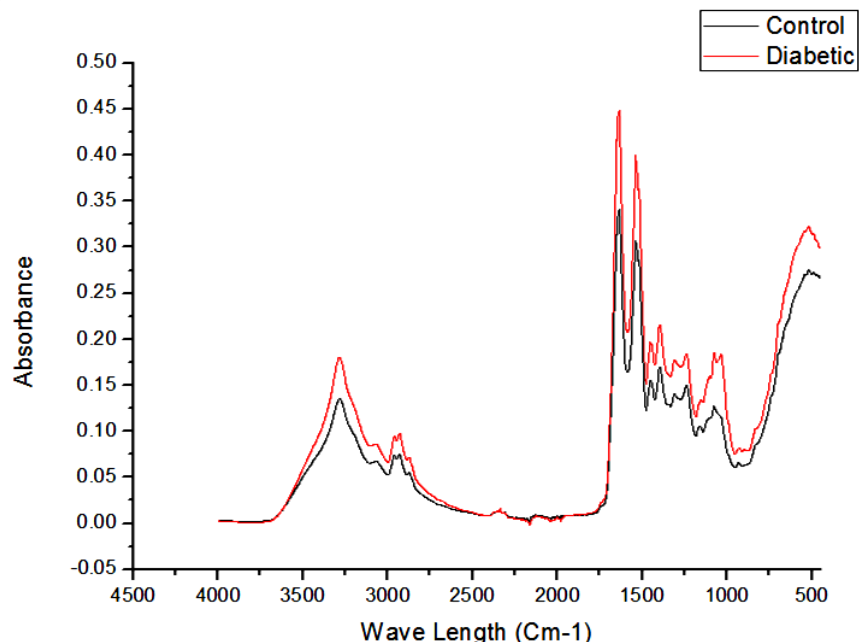
### Results:-

Diabetes mellitus is group of metabolic disorders characterised by hyperglycemia, glycosuria and hyper lipidemia. DM accompanied not only by a permanently elevated level of blood glucose and altered levels of other biomarkers, but also by changes in the conformation of blood plasma proteins and other bio molecules associated with the pathogenesis of diabetes. The variations in biochemical composition of experimental rats induced with alloxan is given in table 1. The results shows that levels of glucose highly significant ( $P<0.001$ ) among control and experimental animals. Further lipid profile found to be moderately significant between control and diabetic rat. But statistical significant values of thyroid hormones were obtained on control and rat induced alloxan . But other biochemical parameters shown in the table 1 are is not significant.

**Table 1:-** Changes in biochemical composition levels in Blood serum of healthy control and Alloxan induced male wistar rat

Biomolecules	Control	Alloxan induced	Statistics
T3(ng/dl)	161 ± 15.87	178 ± 8.19	NS
T4(μ/dl)	5.9 ± 1.20	6.9 ± 1.09	NS
TSH (mIU/dl)	4.8 ± 1.33	5.2 ± 3.11	P<0.05
Plasma Glucose (mgs/dl)	112 ± 5.42	489 ± 8.48***	P<0.001
Total Cholesterol (mgs/dl)	167 ± 3.10	219 ± 3.82**	P<0.01
Triglyceride(mgs/dl)	120 ± 3.89	145 ± 4.07*	P<0.01
HDL Cholesterol (mgs/dl)	47 ± 3.87	58 ± 3.90*	P<0.01
Urea (mgs/dl)	30 ± 7.10	33 ± 7.20	NS
Creatinine (mgs/dl)	0.7 ± 0.4	0.76 ± 0.5	NS
Uric acid (mgs/dl)	4.8 ± 1.1	4.9 ± 1.3	NS
Calcium (mgs/dl)	7.9±1.4	8.0±1.7	NS
Total Protein (gms/dl)	7.1±2.89	7.3±2.96	NS
Albumin (gms/dl)	5.1±2.11	5.3±2.02	NS
Globulin (gms/dl)	2.0±1.0	2.3±1.09	NS
SGOT (IU/l)	55±5.1	58±4.8	NS
SGPT(IU/l)	61±7.5	60±11.4	NS
SAP(IU/l)	121±12	133±15.1	NS

### Serum FTIR Vibration Band Assignment :



**Fig.1:-** Serum FTIR-ATR Spectral pattern of control and diabetes induced by alloxan in Wistar male rat

FTIR spectroscopy measurements were performed on Serum samples obtained from control and DM induced by alloxan in Male Wistar Rat. Figure 1 shows a typical FTIR-ATR spectrum of serum sample, compared with a control and DM induced by Alloxan. The prominent absorption peak  $3283\text{ cm}^{-1}$  is due to the N-H stretching mode (amide A) of proteins. The spectral region  $3072\text{ cm}^{-1}$  comprises of C-H and O-H stretch of lipids of unsaturated fatty acids and N-H stretching vibrations of the Amide B band due to overtone of amide I band. The symmetric/asymmetric stretching vibrations of methyl group of protein and C-H lipids (fatty acids and triglycerides) are found to be present around  $2930\text{--}2875\text{ cm}^{-1}$ . The absorption peaks at  $1743\text{ cm}^{-1}$  corresponds to C=O group of cholesterol ester (HDL). The strong absorption band at  $1634\text{ cm}^{-1}$  corresponds to aryl substituted C=C amide I band mainly due to C=O, C=N and N-H stretch, whereas the vibration at  $1538\text{ cm}^{-1}$  is attributed as amide II band due to NH vibrations stretching coupled with C-N stretching vibrations in protein. The absorption peaks in the region ( $1400\text{--}1300\text{ cm}^{-1}$ ) arise due to the C-H deformation of methyl and methylene group of the proteins, lipids. The asymmetric and symmetric P-O stretching vibrations are found to be around  $1245\text{ cm}^{-1}$  and symmetric P-O stretching of nucleic acid vibrations and ring vibration mode of C-O-H and C-O-C bonds (CO-O-C) asymmetric cholesterol ester; Phosphoric acid are found to be around  $1245\text{ cm}^{-1}$  and  $1165\text{ cm}^{-1}$  respectively. The spectral region  $1115\text{--}1040\text{ cm}^{-1}$  predominantly occupied by C-O characterization and stretching of glucose and glycogen. The ribose and Phospholipids and poly sulfidic S-S stretch in cystic acid vibrations are found to be at  $934\text{ cm}^{-1}$  and  $517\text{ cm}^{-1}$  respectively (Table2).

**Table 2:-** FTIR Vibration Band assignment of blood serum of control and DM induced by Alloxan in Male wistar rat

S.No	Wave Number (cm <sup>-1</sup> )	Vibration Band assignment
1	3283	N-H stretch due to protein and Urea
2	3071	Amide B band due to overtone of Amide I band and olefinic group C-H stretch Lipids of Unsaturated fatty acid
3	2961	C-O-C Asymmetric / Symmetric stretch vibrations of Methyl group of Protein and C-H Lipids (Fatty acids and TGL)
4	2931	Asymmetric stretching vibrations of Methylene group of protein and lipids
5	2879	Symmetric stretching vibrations of Methylene group of protein and lipids
6	1742	C=O group of cholesterol ester (HDL)
7	1634	Aryl substituted C=C Amide I band mainly due to C=O, C=N and N-H stretching
8	1538	Amide II band due to NH vibrations stretching coupled with C-N stretching

		vibrations in protein.
9	1453	Asymmetric bending vibrations of lipids, proteins of CH <sub>3</sub> groups.
10	1395	Free Amino Acid and Fatty Acids
11	1313	Amide III erythrocyte
12	1240	Amide III and Asymmetric PO <sub>2</sub> stretching vibration mode of Nucleic acid
13	1165	Ring vibrational mode of C-O-H and C-O-C bonds (CO-O-C) asymmetric Cholesterol ester; Phosphoric acid
14	1115	Stretching vibration of glycogen
15	1076	C-O characterization stretching of glucose
16	1040	Primary alcohol C-O stretch glucose-Muco Poly saccharide
17	934	Ribose, Phospholipids
18	532	Polysulfidic S-S stretch in cystic acid

### Internal Standard ratio Parameters calculation:-

These spectra were used in Internal ratio Parameter calculation and analysis requires spectra with change in sensitive peaks and no change in sensitive peaks for control and experimental animals. Internal ratio parameter is calculated to fortify the results obtained from the FTIR intensity of absorptions. Internal ratio Parameter ignores the difference in the amount of sample analyzed, it nullifies the contradiction in the quantity of the sample and gives measured out exact deviations in the male wistar ratio ( $I_{3282/1453}$ ,  $I_{2961/1743}$ ,  $I_{1537/1238}$  and  $I_{1075/516}$ ). The internal ratio parameter of basic protein, lipid, Nucleic acid and glycogen of control and diabetic rats given in Table 3. The results shows that peak ratio for NH (protein and Urea) to Lipoproteins-(CH<sub>3</sub>)<sub>asym.bending</sub> among diabetic status of rat induced by alloxan (0.9152) and control male wistar rats (0.8707) is negligible. Significant elevation in the absorption peak ratio was documented in (Protein)<sub>asym and Sym</sub> to TGL/HDL- cholesterol ester in diabetic induction on rat by alloxan and control healthy rat. Further, slight variation peak absorption ratio for Amide II to Amide III and Nucleic acid-PO<sub>2</sub> as well as Glucose Stretching to S-S stretch in cystic acid were noticed in control and experimental rats.

**Table 3:-** Internal Standard ratio Parameters calculation of Lipids, Proteins and Glucose between control and Diabetic blood serum of male wistar rat.

Peak ratio	Wave Number (cm <sup>-1</sup> )	Absorbance	
		Control	Diabetic Rat
NH (protein and Urea) / Lipoproteins-(CH <sub>3</sub> ) <sub>asym.bending</sub>	$I_{3282/1453}$	0.8707	0.9152
(Protein) <sub>asym and Sym</sub> and TGL /HDL- cholesterol ester	$I_{2961/1743}$	3.8586	4.1585
Amide II / Amide III and Nucleic acid-PO <sub>2</sub>	$I_{1537/1238}$	2.0555	2.1695
Glucose Stretching / S-S stretch in cystic acid	$I_{1075/516}$	0.4609	0.5727

### Discussion:-

The current diagnostic tools are insufficient for the early detection of many diseases, including type 1 diabetes mellitus. However, the observation of these structural changes by infrared spectroscopy is limited. Therefore, we used FTIR-ATR Spectroscopy which is inherently sensitive to the structure of bio molecules and able to detect stretching of atoms in the functional group of molecules. We investigated the blood plasma samples of diabetic rat and healthy rats using FTIR-ATR spectroscopic method. The results were combined with conventional methods of molecules quantification i.e. ELISA and Spectrophotometer. The obtained data sets were statistically evaluated and focusing on the spectral ranges that correspond to the structure and conformation of proteins and other biomolecules. Our results suggest that FTIR-ATR spectroscopic method gives more detailed information about the structure of bio molecules; and therefore, might be a promising complement to conventional in diagnostic methods. The results obtained shows that elevated fatty acid types and its derivatives in addition to blood glucose level (Table 1) suggest that Diabetes is not a single disease it's group of heterogeneous syndromes and support the study of Patel, D 2011 where the author stated the clinical features such as heart attack, stroke and peripheral vascular disease in addition to diabetic condition. Further the results obtained in this study also support earlier literature shows that diabetes, and higher-than normal blood glucose levels are well-known risk factors for development of type 2 diabetes recent prediction models have incorporated these with other readily measurable features of metabolic syndrome

(elevated blood pressure, low HDL cholesterol, and elevated triglycerides) to generate validated prediction rules (Stern M.P. 2002; Wilson, P.W, 2007, Schmidt, M.I, 2003). In the average FTIR-ATR spectra of diabetic and healthy controls (Fig. 1), we can recognize that the absorbance's for corresponding wave length to diabetes induced rat is significantly higher intense bands than control shows elevated levels of diabetic biomarker which support other methodology adopted in this study. Apart from this, the spectrum obtained for control and experimental model might be the additional supporting evidences to evaluate the bio molecule qualitatively and quantitatively as diagnostic tool in clinical study. The small changes in the absorption is also appreciable in the FTIR-ATR spectra as it depends on the short existing, effective evanescent wave with 0.5  $\mu$ -5  $\mu$  depth of penetrations. The trends observed on absorptions of internal peak ratio of experimental and control male wistar rat in this current study support earlier studies on different diseases like thyroid, Renal, atherosclerosis, cancer, Hepatitis (Khatheer Saira et al., 2016, Kamatchi, S et al., 2016, Renugadevi, T.S.R, et al., 2009, Haas, S, L, et al., 2010, Dimitrova, N, 2009, Mackanos, M.A, et al., 2009 Gunasekaran, S. et al 2008, 2010, Sankari, G, et al., 2010).

### Conclusion:-

Diabetes mellitus has been induced by administration of Alloxan in dose of 40 mg/kg, subcutaneous administration (0, 5, 11 and 12<sup>th</sup> days) for four dose. Biochemical blood exams showed an increase of Total cholesterol triglyceride, TSH etc., The results obtained suggested that to rat, subcutaneous administrated alloxan induces diabetes mellitus with clinical and complementary signs, this experiment being useful in experimental studies regarding diabetes mellitus. We have analyzed real clinical blood serum samples by FTIR-ATR spectroscopy and identified spectral regions that are most likely reveals the qualitative and quantitative evaluation of bio molecules. The subsequent multivariate analysis of spectral data proved that the FTIR-ATR spectroscopic methods are able to detect more complex signal of serum bio molecules than conventional methods. The results obtained suggested that FTIR-ATR spectral evaluation might be an additional tool in clinical diagnosis, prognosis and disease management. The best predicting model included adiponectin, C-reactive protein (CRP), ferritin, interleukin-2 receptor A (IL2RA), glucose, and insulin, with area under the receiver operator characteristic curve may be suggested for future study.

### Acknowledgement:-

I thank Saveetha Medical College, Chennai and Central Institute of Brackishwater aquaculture (ICAR), Chennai for providing me with all the facilities required in development of this review article.

### References:-

1. Atkinson, M. and Hosking, D. J. Clin. Gastroenterol 12, 633 (1983).
2. Barman, I. . Dingari, N. C Kang, J. W. . Horowitz, G. L Dasari, R. R. and Feld, M. S. Anal. Chem., 2012, 84, 2474-2482.
3. Baulsir, C.F, Simler, R.J. Advanced Drug Delivery Reviews, 21 (1996) 191-203. 949(1949) 137
4. Brands, A. M, Henselmans, J. M. de Haan, E. H and Biessels, G. J. Ned Tijdschr Geneesk. 147, 11-14 (2003).
5. Chu, C. Lin, M. T, Shian, L. R. and Leu, S. Y. Diabetes 35, 481-485 (1986).
6. Cunha-Vaz, J. G. Faria de Abreu, J. R. Campos, A. J. and Figo, G. M. Br. J. Ophthalmol. 59, 649-656 (1975).
7. Dimitrova M, Ivanova, D, Karamancheva I, Mileu, A and Dobreu, I. Application of FTIR Spectroscopy for Diagnosis of Breast Cancer Tumors. Journal of University of Chemical Technology and Metallurgy, 2009, 297-300.
8. Eizirik D.L, Pipeleers D.G, Ling Z, Welsh N, Hellerström, C, Andersson A. Major species differences between humans and rodents in the susceptibility to pancreatic beta-cell injury. Proceedings of the National Academy of Sciences of the United States of America 1994;91(20):9253-6.
9. Enea, N. A Hollis, T. M. Kern, J. A. and Gardner, T. W. Arch. Ophthalmol. 107, 270-274 (1989).
10. Etuk, E.U.N.J. Animals models for studying diabetes mellitus. Agric. Biol 2010;1:130-4.
11. Federiuk, I.F, Casey, H.M, Quinn, M.J, Wood, M.D, Ward, W.K.
12. Induction of type-1 diabetes mellitus in laboratory rats by use of alloxan: route of administration, pitfalls, and insulin treatment. Comparative medicine :252-77
13. Feride Severcan, Ftir spectroscopy as a novel method in characterization and diagnosis of type I diabetes in rat animal model and the protective role of antioxidants. J Diabetes Metab 2013, 4:6

14. Goyal, R. K and . Spiro, H. M. Clin. North Am. 155, 1031 (1983).
15. Grossman, E. and Messerli, F. H. Ann. Intern. Med.125, 304–310 (1996).
16. Gunasekaran, S, Renuga Devi T.S and Sakthivel P.S. Efficacy of simvastatin on chronic Renal failure patients- A Spectroscopic Approach, Asian Journal of Chemistry.Vol.20.NO.1.2008 pages 167-476.
17. Gunasekaran, S, Uthra, D, Sailatha, E and Anita, B. FTIR Spectral Study on Jaundice Blood Samples before and after Treatment. Asian Journal of Chemistry, 2010 22, 51-56
18. Haas, S.L, Möller, R, Fernandes, A, Dzeyk-Boycheva K, Hohmann J, Hemberger S, Elmas, E, Brök- hmann M, Bugert P and Backhaus, J. Spectroscopic Diagnosis of Myocardial Infarction and Heart Failure by Fourier Transforms Infrared Spectroscopy in Serum Samples. Applied Spectroscopy, 2010 64, 262-267.
19. Hassan, A. A, Hassouna, M.M Taketo, T.C. Gagnon and Elhilali, M.M J. Urol. 149(1), 148 – 54 (1993).
20. Iranloye, BO, Arikawe,, AP, Rotimi G, Sogbade ,AO. Anti-diabetic and anti-oxidant effects of Zingiber officinale on alloxan- induced and insulin-resistant diabetic male rats. Nigerian journal of physiological sciences : official publication of the Physiological Society of Nigeria 2011;26(1):89-96.
21. Kanazawa, Y, Kawakami, M, and Kawano, M, New classification of diabetes by ADA and WHO with special reference to pathogenesis. Nippon Rinsho, 57, 551–555 (1999).
22. Kamatchi, S, Gunasekaran, S, Sailatha ,E , Pavithra ,R, Kuppuraj, P . FTIR-ATR Spectroscopic Technique on Human Single Intact Hair Fibre -A Case Study of Thyroid Patients, International Journal of Advanced Scientific Technologies in Engineering and Management Sciences (IJASTEMS -ISSN: 2454-356X), Volume.2, Issue.5,pages1-6 , 2016.
23. Katon,, JE, Micro, 27 (1996) 303-314.
24. Khatheeraja Saira , FTIR-ATR Spectroscopy as Diagnostic tool in Hypothyroidism induced by Carbimazole –A study in Animal model, European Journal of Biomedical and Pharmaceutical Sciences, Volume 3, Issue 12, 362-369, 2016.
25. Klein, R, Klein, B. E, Moss, S. E and Crus- cishanks, K. J. Ophthalmology 102, 7–16 (1995).
26. Lasch P and Kneipp, Biomedical Vibrational Spectroscopy, ed. J John Wiley & Sons, Inc., New York, 2008.
27. Mackanos, M.A and Contag, C.H. FTIR Micro Spectroscopy for Improved Prostate Cancer Diagnosis. Trends in Biotechnology, 2009 27, 661-663.
28. Mitchell, A. L. Gajjar, K. B Theophilou, G. Martin, F. L. and. Martin-Hirsch, P. L J. Biophotonics, 2014, 7, 153–165.
29. Ollesch, J, Drees, . S. L , Heise, . H. M, Behrens, T. Bruning , T. and Gerwert, K Analyst, 2013, 138, 4092–4102.
30. Olsztyńska-Janus, S, Szymborska-Malek K. Gasiór- Głogowska, M. Walski, T, Komorowska, M. Witkiewicz, W. Pezowicz, C.. Kobielaż M and Szotek, S. Acta Bioeng. Biomech., 2012, 14, 101–115
31. Patel D, Kumar R, Prasad S, Sairam K, Hemalatha S. Antidiabetic and in vitro antioxidant potential of Hybanthus enneaspermus(Linn) F. Muell in streptozotocin-induced diabetic rats. Asian Pacific journal of tropical biomedicine 2011;1(4):316-22
32. Petrich, W. Appl. Spectrosc. Rev., 2001, 36, 181–237.
33. Puavilai, G, Chanprasertyotin, S and Sriphrapradaeng, A. Diagnostic criteria for diabetes mellitus and other categories of glucose intolerance:1997 criteria by the expert committee on the diagnosis and classification of diabetes mellitus (ADA), 1998
34. Renuga Devi, T.S, Gnasekaran ,S , Wesley Hudson, J and Sarone Angelah Joybell I. Analysis of renal failure patients blood samples: Characterization and efficacy study. Indian Journal of Science and Technology, Vol.2 No.2, 2002. pages 46-50
35. Sankari, G, Krishnamoorthy, E, ,Jayakumaran, S, Gunasekaran ,V, Vishnupriya, V, Shyama Subramanian, Subramaniam, S and Surapaneni Krishna Mohan. Analysis of serum Immunoglobulin's using FTIR spectral measurements, J. Biology and Medicine , 2 ( 2010)42-48
36. Saud Alarifi , Amin Al-Doaiss , Saad Alkahtani S,A, Al-Farraj, Mohammed Saad Al-Eissa B. Al-Dahmash , Hamad Al-Yahya , Mohammed Mubarak . Blood chemical changes and renal histological alterations induced by gentamicin in rats, Saudi Journal of biological sciences, (2012)19,103-110
37. Schiller, L.R. and Feldman, M. in: Gastrointestinal complications of diabetes, edited by K. L. Becker, Principles and Practice of Endocrinology and Meta- bolism, (Lippincott, JB, 1990), pp. 1144–1147.
38. Schmidt, M.I, Duncan, B.B, Vigo A, Pankow ,J, Ballantyne, C.M, Couper, D, Brancati, F, Folsom, A.R. Detection of undiagnosed di- abetes and other hyperglycemia states: the Atherosclerosis Risk in Communities Study. Diabetes Care 2003;26:1338 –1343



39. Stapleton, S. R. *Cellul. Molec. Life Sci.* 57, 1874-1879 (2000). Stern MP, Williams K, Haffner SM. Identification of persons at high risk for type 2 diabetes mellitus: do we need the oral glucose tolerance test? *Ann Intern Med* 2002; 136:575-581
40. Thomas, W. H. K., Helen, E. T., Leonard, C. H., and Janette, A., The beta cell in autoimmune diabetes: many mechanisms and pathways of loss. *Trends Endocrinol. Metab.*, 11, 11-15 (2000).
41. Thomas, J. M., Contreras, J. L., Smyth, C. A., Lobashevsky, A., Jenkins, S., Hubbard, W. J., Eckhoš, D. E., Stavrou, S., Neville Thomson, A. B. *Diabetes.* 32(10), 900-907 (1983).
42. Tranquilut ,N.C , Tranquilut M.A.C, Torres E.B., Rosario , J.C,Reyes, B.A.S; Hypoglycemic effect of *Lagerstroemia speciosa (L)* Pers. On alloxan -induced diabetic mice . *J.Med. Plants Res.* 3 ,1066-1971(2009)
43. Tyrberg, B, Andersson A, Borg L.A. Species differences in susceptibility of transplanted and cultured, pancreatic islets to the beta-cell toxin alloxan. *General and comparative endocrinology* 2001;122(3):238-51
44. [Wahba, Z. Z and Soliman, K. F](#) *Experientia* 44, 742- 746 (1988).
45. Wilson, P.W, Meigs, J.B, Sullivan L, Fox, C.S, Nathan, D.M, D'Agostino R.B S. Prediction of incident diabetes mellitus in middle- aged adults: the Framingham Offspring Study. *Arch Intern Med* 2007;167:1068 -1074