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Screening of Diabetes Mellitus Induced by Alloxan in Wister Rat Using FTIR - ATR Spectral , Biochemical and Histological Studies Approach

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ABSTRACT
Screening of diseases in the medicine plays vital role in identification , treatment and management of diseases. Though we have numerous methods for screening Diabetes Mellitus and each methods has its own specificities concern with principles and technologies adopted. In this current research work, FTIR ATR techniques was adopted to screen the Diabetes Mellitus along with biochemical and histological analysis as comparison and supportive. Experiments were conducted on Diabetes Mellitus induced Wister rat with alloxan for 30 days followed by termination with analyzing Biochemical, FTIR-ATR spectral and histological studies . The results obtained were more significant for glucose, Triglycerides cholesterol , HDL cholesterol were at $p<0.0001$ level and incase of TSH was significant at $p< 0.05$ level , the other parameters studied were statistically not significant.

The FTIR-ATR study shows that successful spectral differentiation for control and diabetic sample obtained between 3283 – 532 cm-^{1} . The Significant elevation in the absorption peak ratio was documented in (Protein)$_{asym\ and\ Sym}$ to $_{TGL}$ / HDL- cholesterol ester in diabetic induction on rat by alloxan. The slight variation peak absorption ratio for Amide II to Amide III and Nucleic acid-PO2 as well as Glucose Stretching to S-S stretch in cystic acid were noticed. FT-IR spectral results in different tissues of Alloxan induced diabetes shows the intensity of the band at 1040 cm^{-1}, which is due to oligosaccharides,

Histological studies shows thickening of parietal layer of Bowman's capsule of kidney, inflammation in thickened alveolar septae and blood vessels of lungs but in case of liver muscle and heart no appreciable changes recorded .The importance of FTIRATR spectroscopic technique in screening of Diabetes Mellitus is discussed and recommended as diagnostic tool in medical field.

Keywords : FTIR-ATR spectroscopy, Alloxan, Diabetes Mellitits, wistar rat.

INTRODUCTION

Screening identifies a possible disease or disorder and can be carried out by various studies. The procedure may involve various techniques and procedures to identify the cause of an illness or disorder. Further, used to screen for a disease or to provide early disease identification; to diagnose a disease; to provide prognostic information by assessing the degree of disease progression or severity; to assist in selecting drugs or targeting medical treatment; and to monitor the course of a disease or condition. Different studies were applied to study the nature of bio molecules via spectrophotometer, electrophoresis, chromatography and immunoassay, enzyme linked assay etc.,. with its respective principles, methodologies, reagents etc., and its costs varies accordingly. Further these conventional methods were overcome with FTIR-ATR spectroscopy methods with greater sensitivity and also facilitate early detection of diseases with viable as alternative additional tool in clinical analysis or screening of diseases

Diabetes Mellitus is caused by high blood glucose levels due to decreased secretion or effectiveness in function of insulin and metabolic disturbances that lead to chronic, irreversible damage to vital organs and systems [1] . Type I diabetes, the insulin- dependent diabetes mellitus (IDDM), is characterized by the autoimmune destruction of pancreatic β cells [2-4] and Type II , the non-insulin- dependent diabetes mellitus (NIDDM), is characterized by target organs, such as liver, muscle, and adipocytes. Complications DM can give rise to severe damage to these organs with affecting the brain [5-7], eye causes diabetic retinopathy [8-10] , cardiovascular system , stomach, gastrointestinal, respiratory system , gall bladder and the reproductive system [11-16]. Earlier literature studies focused on the quantitation of clinically relevant biomarkers present in blood, plasma and/or urine including glucose, electrolytes, proteins, lipids, hormones etc., [17,18]

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and organ [19] .

The functional and pathological abnormalities seen in diabetes in clinical as well as animal experimentation continued to understand the exact molecular mechanism of diabetes [20]. Previous literature shows that Raman and infrared (IR) spectroscopy are powerful for medical diagnostics techniques was adopted for clinical diagnosis [21,22].

Currently , FTIR together with chemo metric methods play important in the field of pathology and diagnosis of disease . Since the 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 in early disease stage. The study on biochemical composition evaluation by spectroscopic techniques can be used not only for understanding the biological nature of the disease, but also for the diagnosis of the disease. The constitution of body fluids have highly specific functional groups and because of their molecular structure , analysis of these body fluids/tissues helps in diagnosing mechanism diseases. The literature surveys shows that spectroscopic studies on both blood parameters and organs in various disease are very much limited. To achieve this, current research study focused on 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 is non-toxic urea derivative with chemical name of alloxan is 2,4,5,6 tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone, which is an oxygenated pyrimidine derivative to the human β cells even in very high doses. It has been widely used to induce experimental Type 1 diabetes in animals such as rabbits, rats, mice and dogs [23-25] and causes selective necrosis of the β- cells of pancreatic islets [26] .

MATERIALS AND METHODS

INDUCTION AND EFFICACY STUDY OF DIABETES MELLITUS ON WISTAR RAT

Alloxan was obtained from Sigma Chemicals Co., St. Louis, MO, USA and 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. Alloxan was dissolved in normal saline and always prepared freshly. 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 [27] . Diabetes Mellitus was induced by using subcutaneous injection of alloxan (100 mg/kg) in 4 doses (0,5, 11 and 12^{th} day) as per the procedure reported [28] . On 14^{th} day of the experiment 3 animals (50% population) were sacrificed by decapitation from the induction group. The blood glucose concentration was measured after collecting blood samples 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 [29,30] . The blood serum and different organs were collected for Biochemical, FTIR – ATR spectral and histological studies

EXPERIMENTAL DESIGN

The wistar rats weighing about 100-150 g were employed in this study were housed and maintained at Animal House of the Saveetha Medical College, Saveetha University, Thandalam Chennai, India. The experiments were carried out with the guidelines approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) as well as Institutional Animal Ethical Committee. The animal house was maintained at an average temperature (24.0^{0} C± 2^{0} C) and 30-70% RH, with 12hr. light-dark cycle (lights on from 8.00 a.m. to 8.00 p.m.). These experimental rats received humane care and were fed with commercial pellet diet and these animals were acclimatized for one week before the start of the experiment. Wistar rats are randomly divided into 2 groups of 6 rats each were housed in polypropylene cages (32.5 ×21×14) cm lined with raw husk which was renewed every 48 hours Group-1 will serve as the normal control and receive distilled water. The second group (Induction study), the experimental animals treated with Alloxan.

BLOOD

At the termination of experiments, the wistar rats were fasted overnight followed by withdrawn of blood samples and collected in plain and EDTA from the heart under mild anaesthesia before killing. Plasma and serum were separated by centrifugation at 3000 rpm for 15 minutes. FTIR-ATR Spectral Analysis the serum samples were properly preserved in ice bags and immediately transported to the wet lab for spectral studies .

Organs:

The experimental rats were killed for excision of organs like , Heart, Liver, Lung , Kidney Muscle, etc., and washed with saline and refrigerated for lyophilization . Lyophilization of organs tissue were done by Scanvac,

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cool safe, 55-9 Denmark vacuum concentrator at Central Institute of Brackish water Aquaculture ,Indian Council of Agricultural Research, Govt. of India , Chennai . Further, the freeze dried samples grounded to powder using mortar and pestle preserved in desiccators containing silica gel till FTIR-ATR spectral analysis. For histological study, the different organs were dissected out, washed with saline and fixed in 10% formalin for histological studies .

BIOCHEMICAL ANALYSIS

The quantitative analysis of blood as well as tissues of different organs is a major field in the clinical chemistry, and its composition is the preferred indicator with respect to the patho physiological condition . The blood serum were analyzed for biochemical parameters including glucose, urea, creatinine, Calcium, phosphorus, uric acid, SGOT, SGPT, total protein, albumin, cholesterol, triglyceride and HDL concentrations by enzymatic assay method using respective commercial diagnostic kits and serum total T_4 ,T_3 and TSH concentration were determined by ELISA method (detection kits provided by Transasia , Zemun, SCG) in a reputed clinical laboratory in Chennai.

FTIR-ATR SPECTRAL MEASUREMENTS

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. FTIR-ATR Spectral Analysis of blood serum and lyophilized of different organs like heart, liver, lung, muscle , and kidney of healthy control and experimental wistar rat 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). The ATR- FTIR spectroscopy is based on the phenomenon known as Total Internal Reflection (TIR) [31,32] . This radiation strikes the interface between the IRE and the serum and tissue 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 and lyophilized tissue held in contact with the crystal. This evanescent wave protrudes only a few microns (0.5µ-5µ) 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 [31,32].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.

Experimental serum Samples were analyzed for spectral recordings in the mid IR region of 4000-450 ^{cm} 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. Serum sample was placed on the IRE crystal and the 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.

HISTOLOGY

Tissues Samples of Organs including Lung, Heart, Liver, Kidney Muscle, etc., after excision from the animals, washed with normal saline to remove blood, fixed in 10% buffered neutral formalin for 12hours. The formalin fixed tissues were dehydrated in ascending grades of ethyl alcohol, cleared in xylene [33] and embedded in paraffin wax. Sections of 5 µm thickness [34] were cut by rotator microtome. At least 25 tissue sections for each organ were assessed. The sections were processed and passed through graded alcohol series, stained with haematoxylin and eosin [35] cleaned in xylene and cover slipped in DPX. Histological examination was done under 10 X magnification using Trinocular Reseach zeiss Microscope] (Gottingen, Germany) and further obtained from 10 random microscopic fields per animal at X 45 and X 100 objective.

STATISTICAL ANALYSIS

The statistical analysis were performed using Statistical Package for Social Science (SPSS, version 17) for Microsoft windows. The data were expressed as Mean and SD and 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. The obtained data sets were statistically evaluated and focusing on the spectral ranges that correspond to the structure and conformation of proteins and other bio molecules.

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Results and Discussion

DIABETES MELLITUS

Diabetes mellitus is characterized by hyperglycemia, glycosuria and hyper lipidemia caused by a shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin and accompanied by a elevated level of blood glucose, altered levels of other bio molecules , changes in the conformation of blood plasma proteins etc., The effect of diabetes is seen on a variety of tissues leading to important secondary complications such as kidney failure, liver dysfunction, cardiac disorders, etc.,. In the present study Fourier Transform Infrared Spectroscopy was used to examine the effects of alloxan -induced diabetes mellitus on the structural components at molecular level.

INDUCTION STUDY

The induction of diabetes mellitus could be achieved by alloxan to induce diabetes mellitus in this study due its low mortality rate and high tolerance by the experimental animals than other diabetogenic agents [36] . Also, it could be given easily by different routes and its diabetogenic action was rapid and permanent as it destructed the beta cells of islets of Langerhans [37]. Several workers have reported that STZ and alloxan induced diabetes mellitus and insulin deficiency lead to increased blood glucose [38] and association between specific diabetic complications and disturbances in various tissues, such as diabetic nephropathy and cardiovascular diseases, but only limited data is available on the possible association between diabetic complications and liver function

QUANTIFICATION OF BIO MOLECULES IN BLOOD SERUM BY ROUTINE METHODS

The current diagnostic tools are insufficient for the early detection of many diseases, including diabetes mellitus. The findings of this study indicate that exposure to inducing agents are capable of inducing adverse significant blood chemical changes in the disease induced wistar rats...The results obtained in this study also support earlier literature shows that diabetes, and higher blood glucose levels (489 mg/dl ± 8.48) than control animal (112 mg/dl ± 5.42). Models have incorporated these with other readily measurable features of metabolic syndrome , low HDL cholesterol (58 mg/dl ± 3.90), and elevated triglycerides (145 mg/dl ± 4.07) and cholesterol (219 mg/dl ± 3.82 , LDL [39-41] . Further parameters analysed were slightly high than control healthy animal and found to be statistically non significant .

Alloxan rapidly and selectively acting on pancreatic beta cells [42] , to induce DNA strand breaks in isolated rat pancreatic islets [43] , also cause massive reduction of the beta cells of islet of Langerhans induce hyperglycemia and elevation of local free radicals in the cell after increasing free radicals in other body organs [44] .

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. The lipid profile is moderately and statistical significant values of TSH was obtained on control and rat induced alloxan . The biochemical parameters like T3,T4, urea, creatinine, uric acid, liver functioning parameters shown in the table 1 are not significant.

FTIR –ATR BIOCHEMICAL VARIATION IN BLOOD SERUM

The results of this current study further revealed the sensitivity of FTIR spectroscopy in precise as well as early diagnosis of diabetes. FTIR-ATR spectral variations (Table 2) and quantification of bio molecules among control and experimental animals(Table 1) observed are statistically more significant.The IR spectroscopy has been successfully attempted for characterizing bio molecules like proteins, carbohydrates, peptides, lipids, biomembrane, pharmaceuticals products , foods , plant , animal tissues etc., Therefore, we investigated the blood plasma samples of diabetic rat and healthy rats using FTIR –ATR spectroscopic method. FTIR Vibration Band assignment of blood serum of control and DM induced by Alloxan in male wistar rat are shown in Table 2. For blood samples , the glucose concentration was proportional to the difference between the values of the second derivative spectrum at 1076 and the author report glucose spectrum at 1082 cm^{-1} and 1093 cm^{-1} agree with present study[.45]

Present study shows that successful spectral differentiation for control and diabetic sample between 3283 – 532 cm^{-1} and recorded between 3050-199 cm^{-1}[20] .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 [46] where the author stated the clinical features such as heart attack, stroke and peripheral vascular disease in addition to diabetic condition

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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 trends observed on absorptions of internal peak ratio of experimental and control wistar rat in this current study (Table 3) support earlier studies on different diseases like thyroid, Renal ,atherosclerosis, cancer and Hepatitis [48-54].

INTERNAL STANDARD RATIO PARAMETERS CALCULATION FOR SERUM

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. 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 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-$(CH3)_{\text{aym.bending}}$ 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 $(\text{Protein})_{\text{asym and Sym}}$ to $_{\text{TGL}}$ /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-PO2 as well as Glucose Stretching to S-S stretch in cystic acid were noticed in control and experimental rats.

FTIR –ATR BIOCHEMICAL VARIATION IN ORGANS

This study reports the applications of FT-IR spectroscopy in diagnosis of Alloxan induced diabetes in different tissues, such as rat kidney, Liver, lung, heart , muscle, etc., . The differentiation of diabetic tissues from control ones was achieved using on spectral differences in different spectral regions. It is known that pathological conditions induce significant alterations in macromolecular content, concentration, structure and dynamics in tissues and membranes

From the mean spectra of diabetic and control experimental are illustrated in Fig. 2 (a-e) one can easily observe that, the intensity of the band at 1040 cm^{-1}, which is due to oligosaccharides increases. This indicates an increase in oligosaccharides concentration in diabetic membranes and similar reports was documented [55] where he observed intensity of band at 1042 cm^{-1}. The spectral parameters investigated that muscle, which is composed of a high content of type I fibers, was found to be more severely affected from diabetes [56] which has disagreement with the present study and this is because the current study did not focus on type I fibers.

Internal peak ratio absorbance of $(\text{Lipoprotein})_{\text{sym /}}$ and HDL choletsreol ester (I 2931/ I1742), $(\text{Lioprotein})_{\text{sym.}}$vib Mucopoly and Glu-str. (I_{2879} /I_{1040}), Amide II and $(\text{Lipoprotein})_{\text{asym}}$.vib. ($I_{5361}$/ I_{1453}), Mucopoly-Glu-str. and $(\text{Chol.estr})_{\text{asym}}$-PO4 ($I_{1240}$ /I_{1165}) are clearly shows that there is marked improvement in the chemical composition changes as a result of silymarin treated compare to alloxan induced DM wistar rat organs like kidney, liver, and lung of experimental animals and statistically these values obtained are highly significant . But the heart and muscle does not show any significant FTIR-ATR spectral changes on the chemical composition among DM induced and treated and indicates muscle and heart are not affected much during the experimental period (Table 4). Though the slight FTIR –ATR variations observed in this study might be due to prolonged exposure of both alloxan and metformin for the male wistar rat.

HISTOPATHOLOGY STUDIES

FT-IR spectroscopy together with microscopy has been used previously to study diabetes-induced alterations in tissues of animal models [57] . The results reveal the power and sensitivity of FT-IR spectroscopy in discriminating diseased and healthy tissues, which will further contribute to the use of FT-IR spectroscopy in disease diagnosis in the field of diabetes (Fig 3 a-e). The earlier literature studies shows that the effects of diabetes on different organs and tissues for longer periods of time (up to 18 months) using other methods than FT-IR spectroscopy [58] and is contrast with current study which demonstrates the effects of a relatively early onset of diabetes with FTIR –ATR method.

The results of both Hypercellular glomeruli with sclerosis , mesangial proliferation.distal tubules showing

vacuoles in cytoplasm of epithelial cells and thickened blood vessels also noticed . Further Thickening of parietal layer of Bowman's capsule observed (Fig. 3ai- a vii) and these results were support the earlier findings of early and late stages of diabetes mellitus 59-61]. But .the other studies shows that the neonatal STZ- (100 mg /kg) induced type –II diabetes alteration in the structural integrity of the apical membrane of proximal tubules of the kidney tissue in the diabetes rats were observed [62] . Further these authors documented in their study about the fatty liver and altered liver microsomal membranes [63-65]. The present study, a yellowish brown color of the liver was observed which could be due to the fatty liver formation which support earlier research finding [66] and he reported that fatty liver and hyperlipidemia in IDDM of streptozotocin induced. Further, findings of present study are in agreement with the findings showed dilatation of veins, loss of usual concentric arrangement of hepatocytes, liver fibrosis and decreased in glycogen activity in their study [67, 68]. Current study observed accumulation of lipid droplets in the cytoplasm of hepatocytes and fhis change was reminiscent to the formation of fatty liver. It could be due to the increased influx of fatty acids into the liver induced by hypoinsulinemia and the low capacity of excretion of lipoprotein secretion from liver resulting from a deficiency of apolipoprotein B synthesis [66] Hyperlipidemia could be another factor for fatty liver formation. Our findings of fatty liver-formation are in agreement with the findings of earlier study [66].

The present study observed that the lungs of diabetic rat induced by alloxan shows inflammation in thickened alveolar septae and blood vessels (Fig 3bi-b iv). Similar observations documented by other author who found alterations in the lung ultra structure when treated with streptozotocin.

The impact of DM on the respiratory tract is characterized by abnormalities in pulmonary function, such as a reduction in lung elastic recoil and in lung volumes, as well as diminished diffusing capacity .Further The earlier research [63-64] documented in their study about the fatty liver and altered liver microsomal (Fig 3. ci-ciii) and similar observations were noticed in this study The other organs tissues like muscles and heart does not show any histological changes with control and alloxan induced experimental wister rat.(Fig 3 d and Fig 3 e)

CONCLUSION

The obtained results suggested that subcutaneous administration of alloxan induces diabetes mellitus with clinical and complementary signs, this experiment being useful in experimental studies regarding diabetes mellitus. The analysed blood serum and different organs samples by FTIR-ATR spectroscopy and Histological study might approach the qualitative and quantitative evaluation of bio molecules to assess biomarker for DM.

Diabetes mellitus has been induced by administration of Alloxan in dose of 40 mg/kg, subcutaneous administration (0,5, 11 and 12th days) for four dose. . Biochemical blood exams showed an increase of Total cholesterol triglyceride , TSH etc., The obtained results suggested that rat subcutaneous administrated alloxan gave 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 revels 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, Cross reactive protein (CRP), ferritin, interleukin-2 receptor A (IL2RA), glucose, and insulin, with area under the a receiver operator characteristic curve may be suggested for future study.

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CONFLICTS OF INTEREST

None

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Table 1. Changes in biochemical composition levels in Blood serum of healthy control b 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	NSs etc.,
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

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^{-1})	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 CH3 groups.
10	1395	Free Amino Acid and Fatty Acids
11	1313	Amide III erythrocyte
12	1240	Amide III and Asymmetric PO2 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 chacterization stretching of glucose

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16	1040	Primary alcohol C-O stretch glucose-Muco Poly saccharide
17	934	Ribose , Phospholipids
18	532	Polysulfidic S-S stretch in cystic acid

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^{-1})	Absorbance	
		Control	Diabetic Rat
NH (protein and Urea) / Lipoproteins-$(CH3)_{aym.bending}$	$I_{3282}/_{1453}$	0.8707	0.9152
$(Protein)_{asym\ and\ Sym\ and\ TGL}$ /HDL- cholesterol ester	$I_{2961}/_{1743}$	3.8586	4.1585
Amide II / Amide III and Nucleic acid-PO2	$I_{1537}/_{1238}$	2.0555	2.1695
Glucose Stretching / S-S stretch in cystic acid	$I_{1075}/_{516}$	0.4609	0.5727

Table 4 The changes in the FTIR-ATR band internal peak ratio calculation for various molecules in the lyophilized organs of control and induced DM experimental rats

Peaks ratio	KIDNEY		p value	LIVER		p value	LUNG		p value	HEART		p value	MUSCLE		p value
	Cont.	DM I		Cont.	DM I		Cont.	DM I		Cont.	DM I		Cont.	DM I	
I_{2931} $(Lipoprotein)_{sym}$/ I_{1742} HDL cholesterol ester	2.7738 ± 0.151	3.2987 ± 0.116	0.001	2.15222± 0.083	2.5608± 0.117	0.0003	2.5389± 0.013	2.9207± 0.0161	0.0000005	2.8611± 0.215	2.8732± 0.164	0.3904	2.4229± 0.102	2.3213± 0.211	0.30
I_{2879} $(Lioprotein)_{sym.vib}$ / I_{1040} Mucopoly-Glu-str.	0.5665 ± 0.012	0.8940 ± 0.116	0.0001	0.7484± 0.020	0.8703± 0.015	0.0000001	0.4114± 0.012	0.5483± 0.0113	0.000007	0.4455 ± 0.022	0.4634± 0.025	0.3632	(	0.6125± 0.040	0.3127
I_{1538} Amide II / I_{1453} $(Lipoprotein)_{asym.vib.}$	0.8024 ± 0.023	1.1346 ± 0.014	0.0001	0.9525± 0.017	1.2674± 0.014	0.0000005	1.5550± 0.012	1.8731± 0.0129	0.0000002	0.9718± 0.017	0.9556± 0.012	0.3505	1. (	1.0004± 0.081	0.3455
I_{1240} Amide III-$_{asym}PO_2$	1.1278 ±	1.3667	0.0001	1.1723±	1.2802± 0.01	0.0013	1.0855± 0.012	1.1510± 0.01	0.0003	1.0507± 0.084	1.0046± 0.087	0.4559	1.0648± 0.07	1.0012± 0.09	0.1202

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of NA	0.054	± 0.018		0.013	3			01					6	5	
I_{1165} (Chol.estr)$_{asym}$-PO_4															

SERUM FTIR VIBRATION BAND ASSIGNMENT

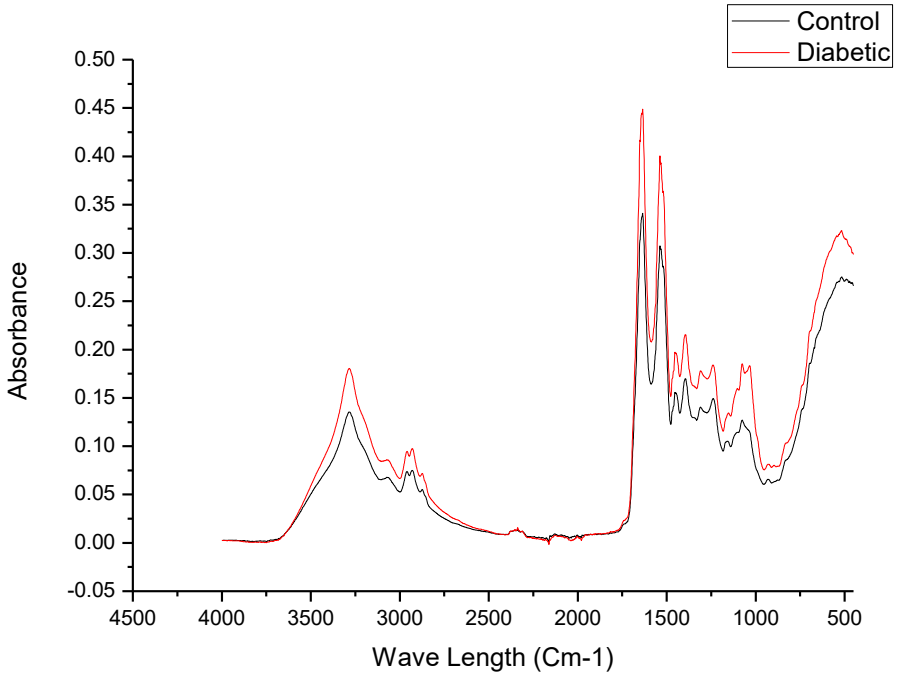

Fig.1 Serum FTIR-ATR Spectral pattern of control and diabetes induced by alloxan in Wistar male rat

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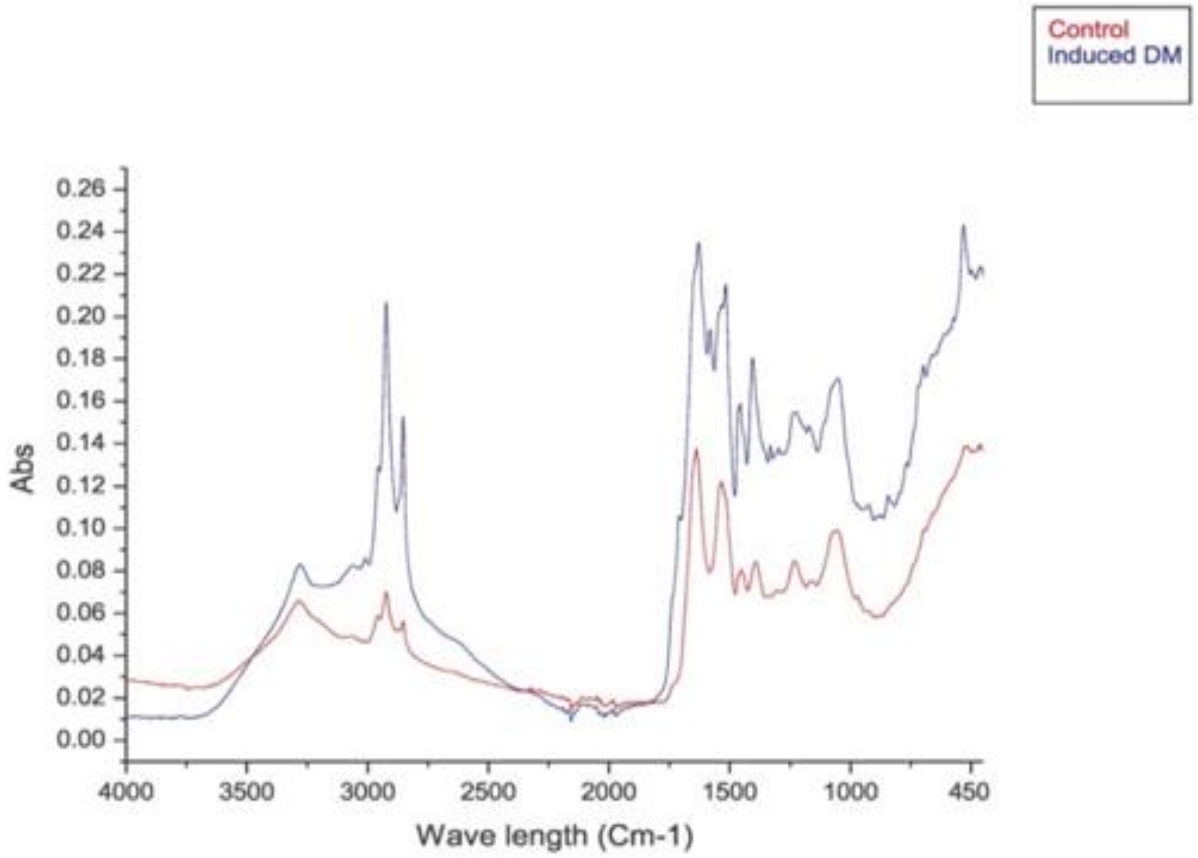

Fig. 2 (a) FTIR-ATR spectral overlaid pattern of kidney of healthy control andalloxan induced diabetes mellitus in experimental male Wistar rat

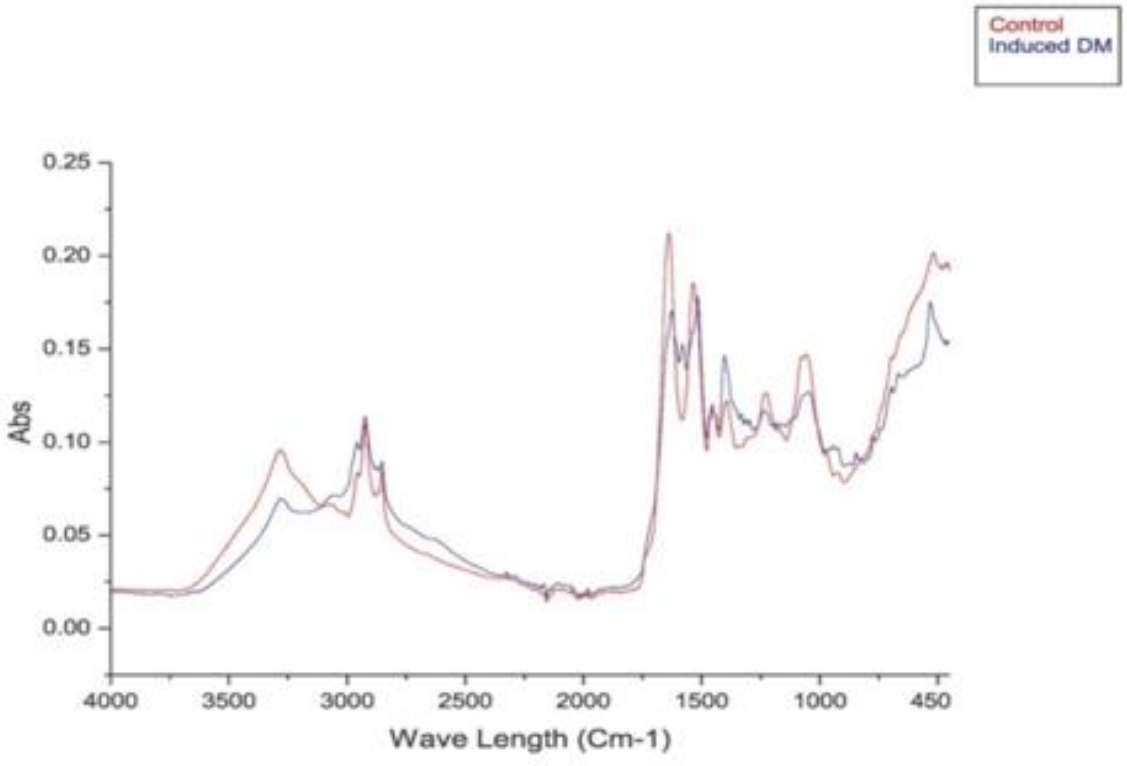

Fig. 2 (b) FTIR-ATR spectral overlaid pattern of liver of healthy control and alloxan induced diabetes mellitus in experimental male Wistar rat

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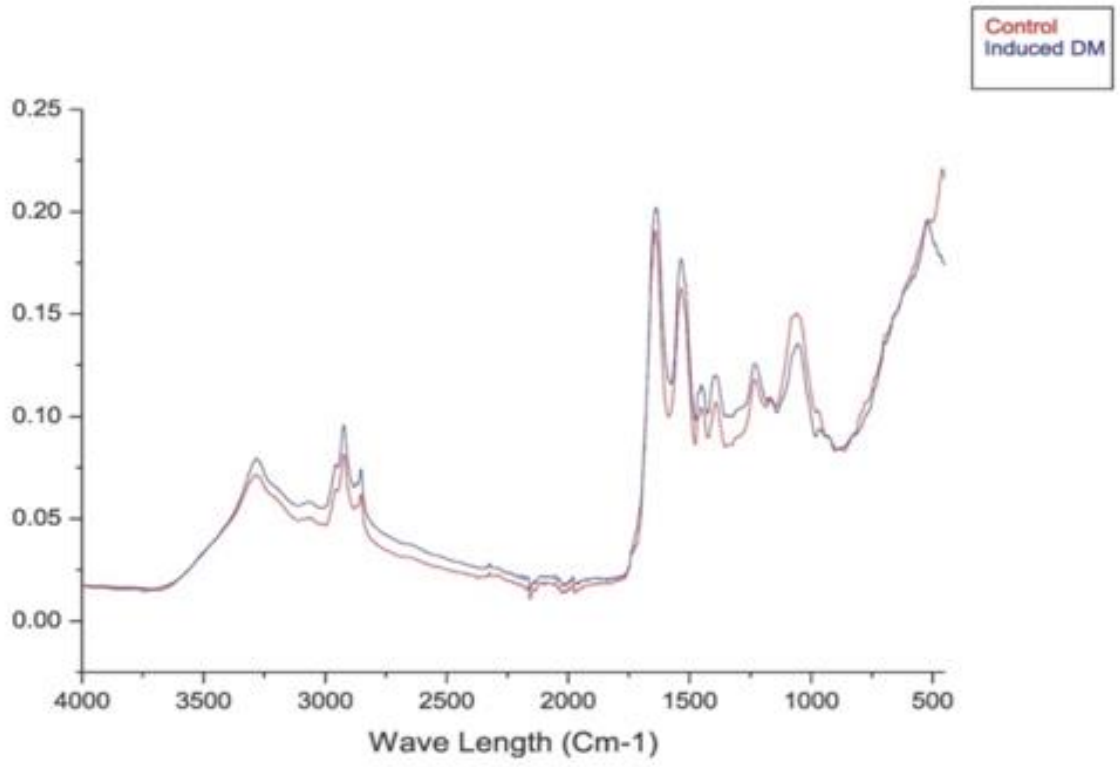

Fig. 2(c) FTIR-ATR spectral overlaid pattern of lung of healthy control and alloxan induced diabetes mellitus in experimental male Wistar rat

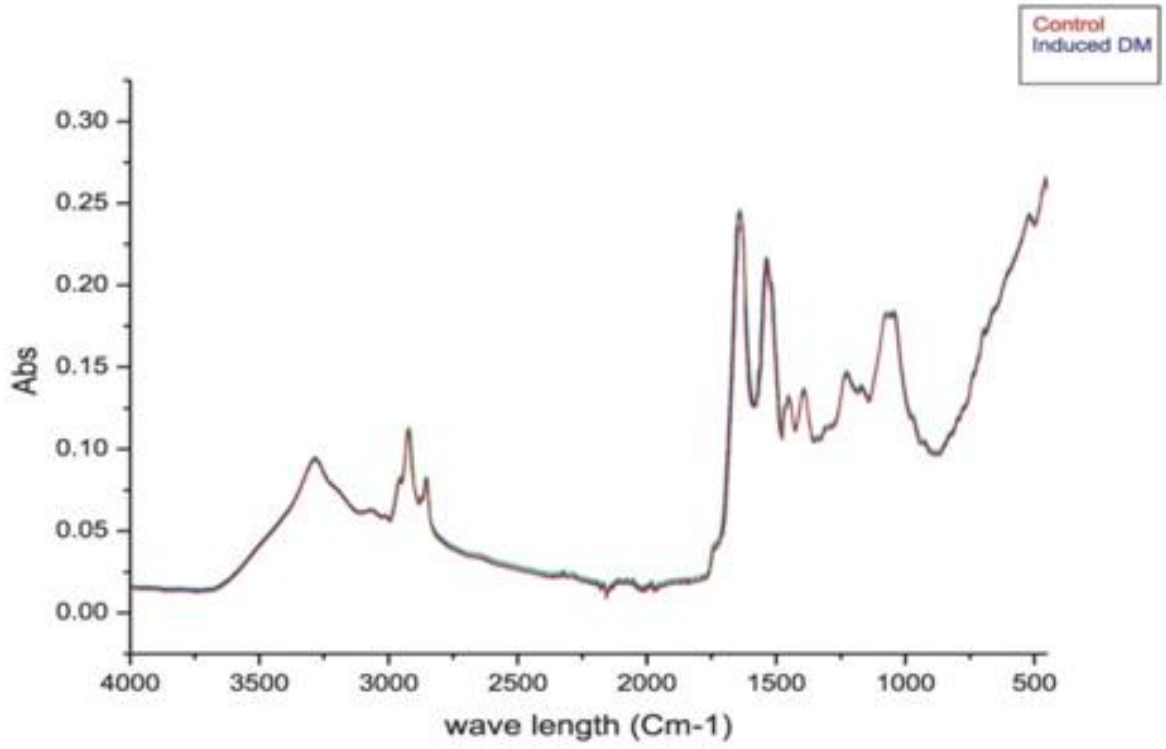

Fig.2 (d)FTIR-ATR spectral overlaid pattern of heart of healthy control and alloxan induced diabetes mellitus in experimental male Wistar rat

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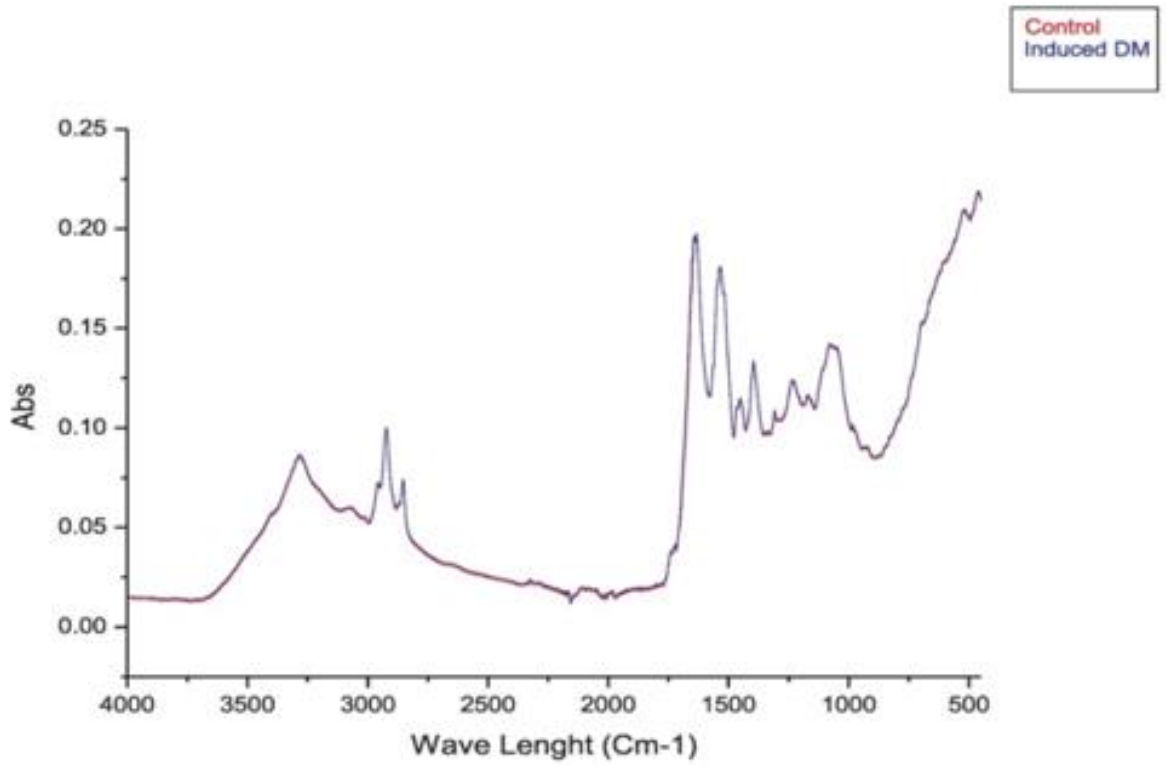
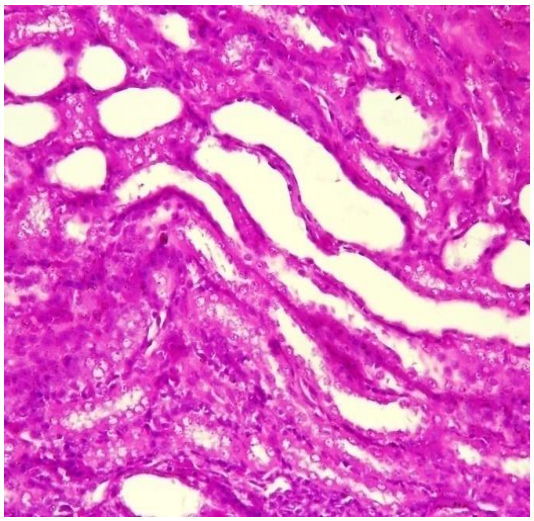
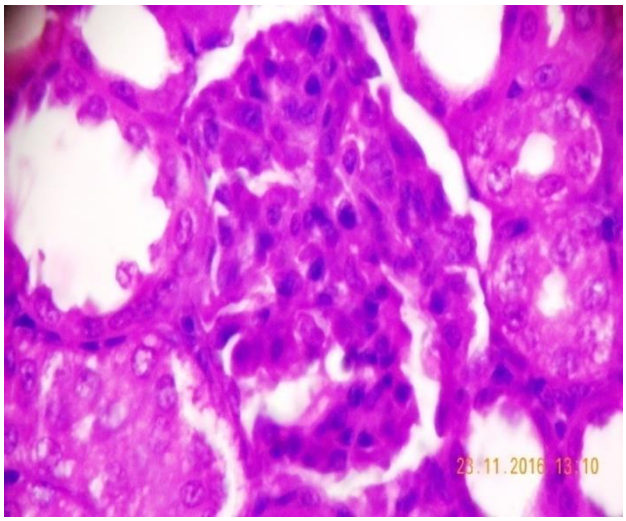


Fig. 2 (e) FTIR-ATR spectral overlaid pattern of muscle of healthy control and alloxan induced diabetes mellitus in experimental male Wistar rat

Figure 3



3(ai) Control group : Kidney -Tubules



3 (aii) Induction- Kidney showing hypercellular glomeruli. H&Ex100 (Induction)

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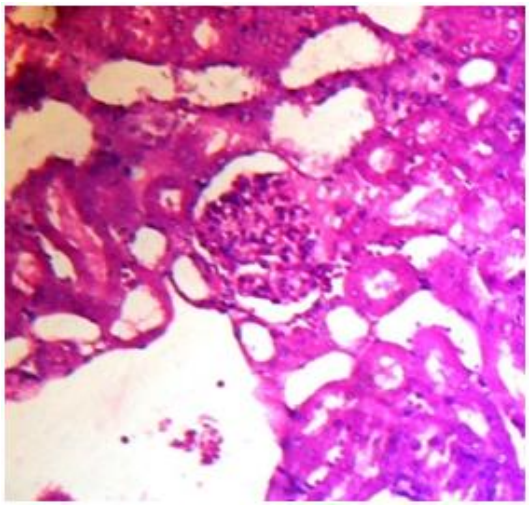
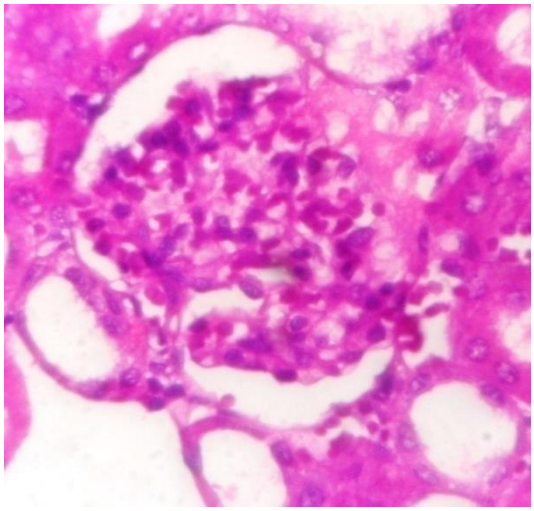
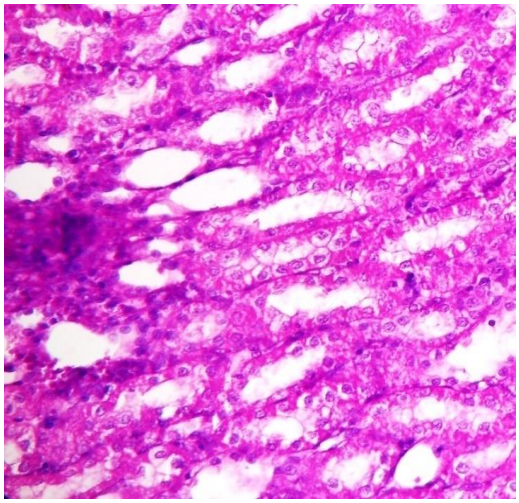


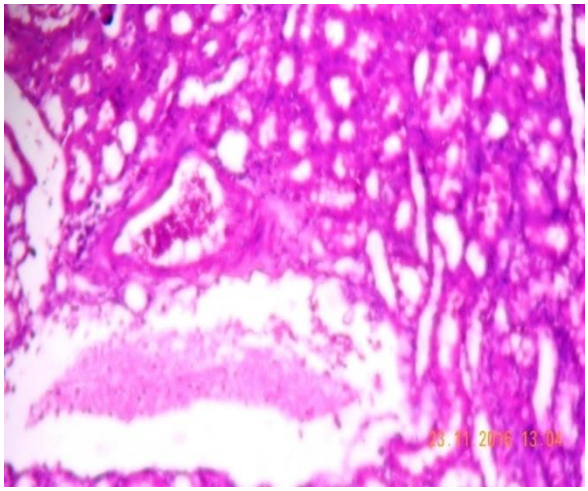
3(aii)Kidney showing mesangial proliferation H&Ex40 (Induction)



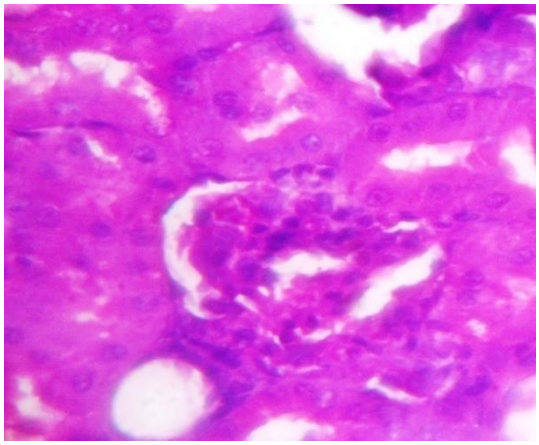
3 (aiv) Mesangial proliferation H&Ex100 (Induction)



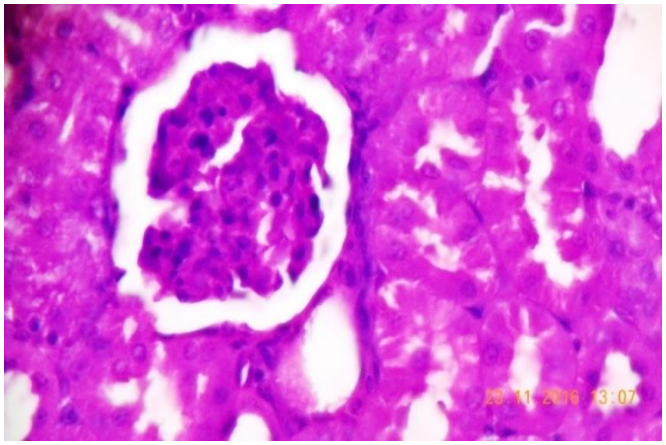
3(a-v)Distal tubules showing vacuoles in cytoplasm H&Ex40 (Induction)



3 (a-vi) Blood vessels with thickened wall H&Ex40 (Induction)



3(a-vii) Glomeruli with sclerosis H&Ex100 (Induction)



3(a-viii) Thickening of parietal layer of Bowman's capsule H&Ex100 (Induction)

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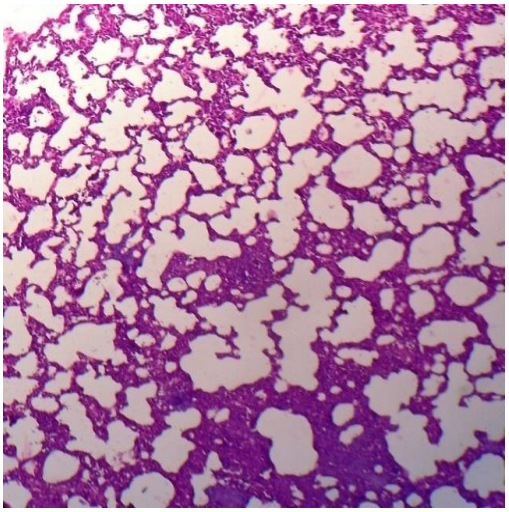
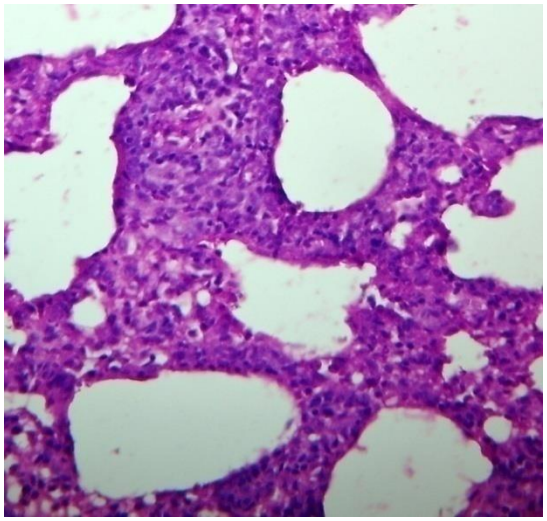
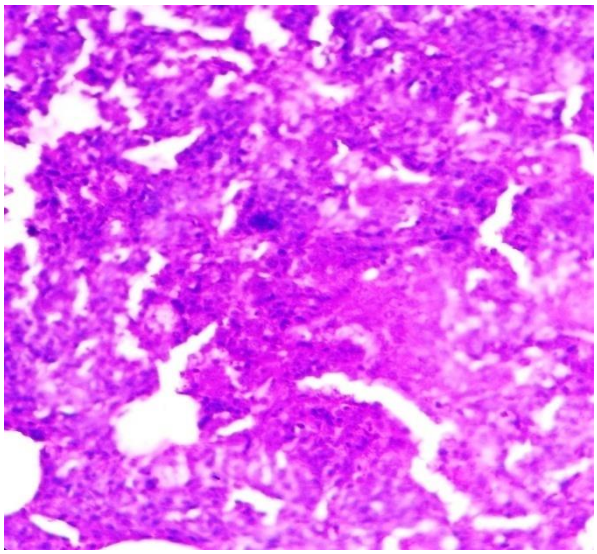


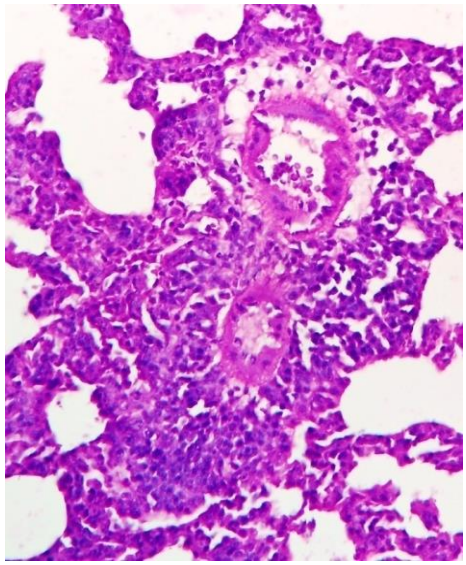
3(b-i) Control group : Lung



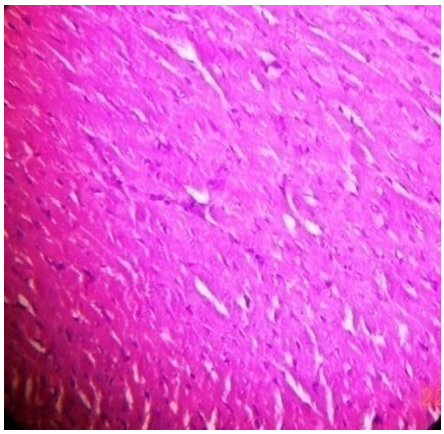
3(b-ii) Lung shows thickened alveolar septae (S) with inflammatory reaction H&Ex40 (Induction)



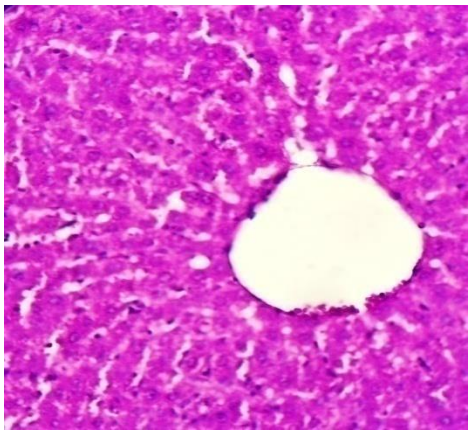
3(b-iii)Marked inflammatory reaction in interstitium



3 (b-iv) Thickened blood vessels H&Ex100 (Induction) and alveoli H&Ex10 –(Induction)



3(Ci) Liver Control group - Normal



3 (Cii) Altered Liver Microsomal H&Ex100 H&Ex100

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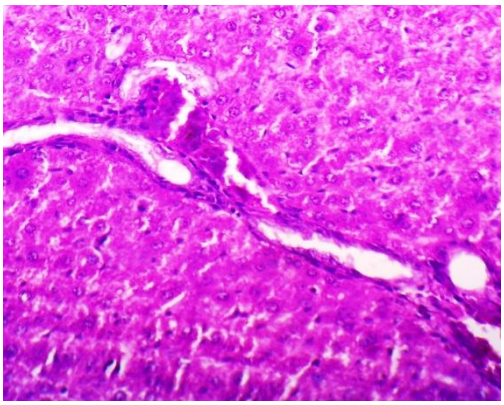
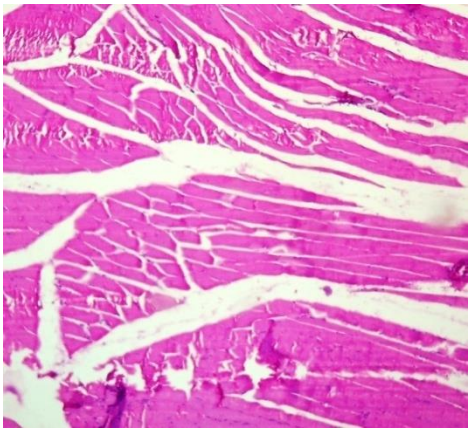
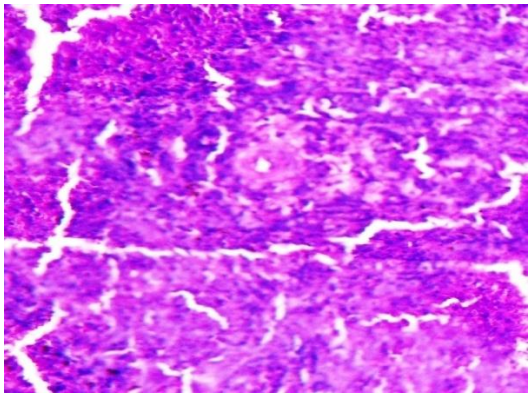


3 (Ciii) Fatty Liver H&Ex100



3(d) Control group : Muscle and DM Induced Muscle H&Ex100- Normal



3 (e)Control group : Heart and DM induced heart -Normal