NMR, LIBS and FTIR based metabolomics of an antidiabetic herbal formulation
Prashant Kumar Rai¹, Rama Jayasundar¹, Gaurav Sharma¹, Ashok Kumar Pathak², Somenath Ghatak³, Geeta Watal³, and A K Rai⁴
¹Department of NMR, All India Institute of Medical Sciences, New Delhi, Delhi, India, ²Department of Physics, University of Allahabad, Allahabad, U P, India, ³Department of Chemistry, University of Allahabad, Allahabad, U P, India

Introduction: Although plants have long been a major source of medicine, there is renewed interest in studying the phytochemistry using a variety of spectroscopic techniques. While the search is for new lead compounds, there is also increasing scientific curiosity about the use of polyherbs which are thought to be multi-targeting and synergistic in action (1). This study reports a comprehensive spectroscopic metabolomics using NMR, Fourier Transformed Infra Red (FTIR) and Laser Induced Breakdown Spectroscopy (LIBS) of a polyherbal formulation, whose antidiabetic activity has also been tested in vivo on rat models.

Materials and Methods: The polyherbal tablet purchased from a GMP certified pharmaceutical company, contains 13 plant ingredients – Bauhinia variegata, Cinnamomum zeylanicum, Cinnamomum tamala, Commiphora mukul, Crataeva religiosa, Ellaria cardamomum, Emblica officinalis, Emblica ribes, Piper longum, Piper nigrum, Terminalia bellirica, Terminalia chebula and Zingerib officinalis.

NMR: Water suppressed 1D proton spectrum was obtained using 700 MHz NMR (Varian) spectrometer. Five gram of the tablet was dissolved in 10 ml of distilled water, centrifuged at 10,000 rpm for 10 minutes at 20°C to settle the non-polar, water insoluble compounds, and then filtered through Whatman’s paper no. 1. Deuterated Trimethylsilyl propionate (TSP) in a coaxial NMR insert served as an external reference. The following parameters were used: spectral width-12 ppm, relaxation delay-2s, no. of scans-64, data points-32 K. NMRshiftDB and SBDS (Integrated Spectral Data Base System for Organic Compounds) data library were used for assignments of the peaks along with 2D NMR data (2).

FTIR: IR spectrum was obtained from the aqueous preparation of the formulation using FTIR 660 (Agilent, USA) in the ATR (Attenuated Total Reflectance). Both mid-IR (4,000 cm⁻¹ to 400 cm⁻¹) and near-IR (-12,500 cm⁻¹ to 4,000 cm⁻¹) regions were studied.

LIBS: The 4-channel spectrometer (Ocean optics LIBS 2000+) equipped with 4-gratings was used to get the dispersed light from the excited sample. The wavelength range of 200-1100 nm was used to record the LIBS spectra at a repetition rate of 2 Hz and 175-mJ-laser energy.

Evaluation of antidiabetic activity in vivo: These studies carried out on rats were approved by the Institutional Ethical Committee. Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) (50 mg kg⁻¹ bw) in 0.1 M citrate buffer (pH = 4.5) to rats fasted overnight. After 3 days of STZ administration, 72 rats with marked hyperglycemia were selected for the study. Blood glucose Level (BGL), Fasting Blood Glucose (FBG), Glucose Tolerance Test (GTT) and Post Prandial Glucose (PPG) were estimated by glucose oxidase method using standard kit (Bayer Diagnostics Ltd., India). The rats were divided into two groups of mild- and sub-diabetes based on their BGL. Four different doses 50, 100, 150, 100 mg kg⁻¹ of formulation were used to identify the most effective dose. The synthetic drug Glibenclamide 5 mg kg⁻¹ was used as a positive control.

Results and discussion
NMR: Figure 1a shows the proton NMR spectrum from the formulation. Resonances from sugars (α and β-glucose), fatty acids, glycerol, amino acids (proline, threonine, tyrosine), organic acids (γ-amino-butyrate, furamic acid, succinic acid, lactic acid) and metabolites such as choline, glycerol, inositol, β-hydroxy butyrate, indoxyl sulphate are observed. The spectrum shows that the formulation is rich in primary plant metabolites (α, β-glucose, glycerol, amino acids), organic and fatty acids and also some secondary (choline and inositol) metabolites. The secondary metabolites play an important role in antidiabetic activity (3).

FTIR: The FTIR spectrum (Fig.1b) shows the following groups: C=C (865-1265 cm⁻¹), C=C (1320-1430 cm⁻¹), NH₂ (1550-1660 cm⁻¹), ketoadhyde C=O (1645-1670 cm⁻¹), NH₂ (2317-2362 cm⁻¹), cycloalkane (2861-2978 cm⁻¹), benzene 750 and 690 cm⁻¹ and a shoulder at 3350 cm⁻¹. Many of these functional groups seen in secondary metabolites are known to have antidiabetic activity (3). Some of the functional groups are also seen in NMR spectrum – eg. OH and CHO as part of carbohydrate structure (α, β- glucose and sucrose), and R-NH₂ in essential amino acids (methionine, proline and threonine).

LIBS: The LIBS spectrum (Fig. 1c) shows the presence of elements such as Mg, Na, K, Ca, O and C in the spectral range 200 - 900 nm. Each element displays several spectral lines corresponding to the different ionisation states. No toxic elements such as Hg and As were observed in the formulation. Free radicals cause disruption in insulin action, mitigate glucose tolerance status and also generate oxidative stress, thus playing a major role in causation of diabetes (4). Ca and Mg are known to quench of free radicals (3) and their presence in the formulation may play a direct role in its antidiabetic activity.

Antidiabetic activity in vivo: The formulation showed antidiabetic activity in both sub- and mild-diabetic rats. The maximum reduction in BGL was observed at 5 mg of Glibenclamide (positive control) in both rat groups. The results also showed that during GTT, the antidiabetic effect of 150 mg/kg of the formulation was comparable to 5 mg of Glibenclamide (positive control) in both rat groups.

Conclusion: This study reports for the first time a comprehensive metabolomic profiling of an antidiabetic polyherbal formulation using three spectroscopic techniques, namely NMR, FTIR and LIBS. The presence of primary and secondary metabolites in NMR and FTIR spectra indicates their possible role in the antidiabetic activity of the formulation. The presence of elements like Mg and Ca in LIBS spectrum, indicate that the regulation of BGL and quenching of free radicals by these elements could play a role in the antidiabetic activity of the formulation.

References: