NMR metabolomics of drug response to antineoplastic polyherbal formulations studied in human hepatocellular carcinoma cells

G. Sharma¹, R. Jayasundar¹, T. Velpandian¹, R. Singh³, S. S. Chauhan¹, V. Kapoor⁴, and S. N. Das⁴

¹NMR, All India Institute of Medical Sciences, New Delhi, India, ²Ocular Pharmacology & Pharmacy, All India Institute of Medical Sciences, New Delhi, India, ³Biochemistry, All India Institute of Medical Sciences, New Delhi, India, ⁴Biotechnology, All India Institute of Medical Sciences, New Delhi, India

Introduction: Cancer is a leading cause of death worldwide and poses a huge health challenge. Among the treatment modalities for cancer, chemotherapy plays an important role. Many of the chemotherapy drugs are plant based and there is continuing interest in evaluating the anticancer properties of medicinal plants (1). There is also a growing appreciation of the use of polyherbal formulations since these are considered to have low toxicity and are also multitargeting (2). The present study has used NMR metabolomics to profile the drug response of human Hep-G2 cancer cells, considered resistant to conventional chemotherapy. The treatment in this study has been administered by four polyherbal formulations, whose antineoplastic activities have also been assessed using apoptosis (programmed cell death) detection assay (3). The cytotoxicity of these formulations on Hep-G2 cells have been previously reported (4).

Materials and Methods: Four polyherbal formulations labeled KG, VK, GTK and MK were selected for study. The formulations were made up of medicinal plants such as Cuminum cyminum, Emblica officinalis, Terminalia chebula, Terminalia bellerrica, Piper nigrum, Piper longum and Raphanus sativus.

Antineoplastic activity: 4 x 10⁴ Hep-G2 cells were plated into each well of 4 sets of 12-well plate and treated for 48 hours with the conventional chemotherapeutic drug paclitaxel (positive control, 10 µg/ml) and 50, 75 & 100 µg/ml of formulations. One set of cells was left untreated. Apoptosis detection assay was carried out using BD Annexin V (A)- Fluorescein isothiocyanate (FITC) Propidium Iodide (PI) apoptosis detection kit and evaluated by flow cytometry (BD LSR II FACS). Cumulative cell death (early and late apoptosis) resulting from treatment was assessed.

Results and Discussion: Of the three concentrations (50, 75 and 100 µg/ml) evaluated, 100 µg/ml showed maximum antineoplastic activity. Amongst the four formulations, VK showed prominent resonances in the spectra caused by citrate, glucose, lactate, oxaloacetate, pyruvate, taurine, L-alanine, L-aspartate, guanido-acetate, methionine, ornithine, proline, glutamic acid, prolyl dipeptide, pyrroloquinoline quinone and trimethylamine. The treatment in this study has produced antineoplastic activity.

Conclusion: NMR profiling of drug induced changes have been demonstrated for the first time for polyherbal formulations, all of which showed antineoplastic activity. Drastic reduction in peaks were observed in 3.5–3.8ppm. This study also provides a first time evidence of polyherbal formulations causing apoptosis in cancer cells.