Proliferative, differentiation and toxicological effects of selected herbal preparations on in house established human mesenchymal and haematopoietic stem cell lines

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ABSTRACT

With the continuous emergence of diseases and disorders, and the incessant search for medications and treatment modalities, drug discovery remains to be a thriving area of research and development. In an era of use of novel technologies in drug screening, the developed world is compelled to collaborate with the developing world to overcome the issue of long-time spans in identification of potential drugs, by extracting time tested traditional medicine knowledge to target different diseases. As Sri Lanka is a country with a rich knowledge of traditional medicine, and is also a biodiversity hotspot, the objective of this study was to investigate on traditional herbal preparations which would identify drug leads for stem cell therapy.

In the current study, human mesenchymal stem cells (hMSCs) and human haematopoietic stem cells (hHSCs) were isolated from umbilical cord tissue and umbilical cord blood, using explant method and $CD34^{+}$magnetic bead cell selection method, respectively. The hMSC line was characterized using immunophenotyping by flowcytometry, trilineage differentiation into adipocytes, chondrocytes and osteocytes and confirming genetic stability of the cells by karyotyping. Genetic stability of the hHSCs were also confirmed by karyotyping. The proliferative effects of selected herbal preparations, *i.e.* mature leaf concentrate of *Carica papaya* (MLCC), *Ficus benghalensis* distillate (FBD) and *Gymnema/Vernonia* distillate (GVD) was performed using the MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay; while genotoxicity was investigated by comet assay, apoptotic effects were screened by acridine orange/ ethidium bromide (AO/EB) assay. Anti-adipogenic assay was performed by quantifying the absorbed oil red O stain by lipid droplets (oil red O dye elution method). The chondrogenic assay quantified the number of cell aggregates in the culture. In addition, to proliferative and differentiation assays carried out with crude MLCC, these were also carried out with the size exclusion chromatography (SEC) fractions and sequential solvent extraction (SSE) fractions of the MLCC. Also, an *in vivo* study on anti-obesity was carried out treating diet induced obese Balb/c mice with crude MLCC based on the promising results of the *in vitro* anti-adipogenic assay. Chemical characterization of SEC fraction 01 of MLCC and FBD which manifested significant bio activity, was carried out using GCMS, LCMS and SDS-PAGE. Crude MLCC showed a plethora of bioactivities; significant hMSC and hHSC proliferation properties on both male and female lines *in vitro*, significant anti-adipogenic activity *in vitro*, significant anti-obesity activity *in vivo* and significant chondrogenic activity *in vitro* ($P<0.05$). Size exclusion chromatography (SEC) fraction 01 of

MLCC showed significant proliferative activity on hMSCs higher than that exerted by crude MLCC ($P<0.05$). Crude MLCC showed no genotoxicity on male / female hMSCs or hHSCs. Chemical characterization of the SEC fraction 01of crude MLCC, evidenced a single non-ionic surfactant and a single protein together with 4 other chemical compounds.

FBD demonstrated significant proliferation activity on male hMSC and hHSC lines but showed significant inhibitory effects on female hMSCs ($P<0.05$) and no significant stimulatory or inhibitory proliferation effects on female hHSCs ($P>0.05$). FBD showed significant inhibitory activity on chondrogenic capacity of hMSCs ($P<0.05$) and no significant activity on adipogenesis ($P>0.05$). While FBD showed significant apoptotic effects on female hMSCs, no genotoxicity effects on male hMSCs or male or female hHSCs were manifested. Cinnamaldehyde and Eugenol were found as major compounds in FBD by GCMS analysis and may be suggested as the responsible compounds for gender based differential proliferation activity on hMSCs and hHSCs.

GVD showed restricted significant proliferative effects on hHSCs, favouring the male cell line ($P<0.05$). GVD showed neither significant activity on chondrogenesis nor on adipogenesis ($P>0.05$). Also, GVD showed no genotoxicity effects on male and female hHSCs.

The results obtained from this study, are suggestive of three prospective natural drug leads with potential use for hMSC and hHSC proliferation, chondrogenic differentiation and anti-obesity. The current study stands strong as a pilot study with preliminary information to carry out in depth research, mechanisms of action at the molecular level, and standardization to produce potential and effective stimulants/drugs for stem cell research and clinical therapy, as alternatives to exorbitantly expensive, existing synthetic and recombinant drugs.