In vitro anti-leukemic activity of *Hemidesmus indicus* and related molecular mechanisms

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Background- Cancer is a highly complex pathology and nowadays an emerging therapeutic approach is represented by the multitarget therapy. This strategy is based on the simultaneous modulation of several targets to contrast the complexity of tumor by different pharmacologically-active agents. In this context, products of natural origin could represent a promising approach, due to their intrinsic complexity and ability to interact with different biological networks (Aggarwal et al., 2009). *Hemidesmus indicus* (HI), belonging to the family of *Asclepidaceae*, represents an Indian weed widely used in the traditional medicine and has been extensively investigated for its pharmacological properties. Several *in vitro* and *in vivo* studies have been conducted to evaluate its anticancer, antioxidant, anti-inflammatory, antipyretic, analgesic, antimicrobial, antidiabetic, hepatoprotective, cardioprotective, renoprotective, neuroprotective and immunomodulatory properties (Das and Singh Bisht, 2012). *Aim*- To evaluate the anti leukemic activity of HI roots decoction in human T lymphobalstoid cell line (Jurkat). Based on initial results, the modulation of key genes responsible for the apoptotic process was screened in terms of protein and gene expression: p53 and Noxa-Mcl-1, an axis of protein involved in p53-independent apoptosis. *Results and discussion*- The results showed the ability of HI to induce apoptosis in a concentration dependent way in Jurkat cells. Concerning the involvement of p53 in the pro-apoptotic action of HI, no significant changes were observed at the protein level, but a dose-dependent down-regulation was observed at mRNA level. P53 could not have a key role in the apoptosis induced by the decoction, thus the research focused on Noxa-Mcl-1.The protein level of Noxa did not show any significant variation under HI treatment, whereas Mcl-1 showed a down-regulation at all tested concentrations, that is contextual to the final effect. Probably, it was not possible to observe the expected Noxa up-regulation because of the lack of a specific antibody for the epitope that Noxa forms with Mcl-1 (Lowman et al., 2010). At mRNA level it was possible to observe an up-regulation of both genes at the highest concentrations of treatment. Reactive oxygen species (ROS) play an important role in the induction of apoptosis. HI induced ROS production after really short time of treatment, and co-treatment with N-acetylcysteine significantly reduced the apoptosis induced by the decoction. This confirms the involvement of the mitochondrial apoptotic pathway in cell death induced by HI. Cytochrome C release and caspase-3 activation strengthened this evidence. *Conclusions*- Based on these results, HI appears as an interesting botanical drugs for its anti-leukemic activity. In this context, it is necessary to underline the importance of therapeutic consistency of botanical drugs. FDA stated that for drug derived from single part of a single plant, as HI, the consistency could be guaranteed by robust chemistry, manufacturing and control measures, 'fingerprinting', and conducting chromatographic analyses of marker compounds (Chen et al., 2008). Our HPLC phytochemical analysis of HI demonstrated the presence of 2-hydroxy-4-methoxybenzaldehyde, 3-hydroxy-4-methoxybenzaldehyde and 2-hydroxy-4- methoxybenzoic acid, which can be used as fingerprint. The phytochemical analysis performed on three batches of HI demonstrated that the levels of upon reported phytomarkers were not statistically different among batches. Of note, the three batches were not statistically different in terms of biological activities.

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