Implementation of the Three Rs in the Human Hazard Assessment of Brazilian Medicinal Plants: An Evaluation of the Cytotoxic and Genotoxic Potentials of Dipteryx alata Vogel

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Summary — In Brazil, medicinal plants are widely used by the indigenous people, which leads to a constant requirement for toxicity tests to be performed on the plant extracts. Although the current Brazilian Directive 90/2004 on the preclinical toxicity testing of phytotherapeutics recommends only in vivo tests, some Brazilian researchers would like to change this situation by implementing the Three Rs in the toxicological testing of medicinal plants. The present study evaluated the cytotoxic and genotoxic potentials of bark extracts from Dipteryx alata Vogel, a medicinal plant of the Brazilian cerrado, by using CHO-K1 (Chinese hamster ovary) cells. An IC50 value was obtained, which corresponded to 0.16mg/ml of plant extract, and from this the equivalent LD50 was determined as 705mg/kg. In order to determine the genotoxic potential of the sample, the frequency of micronucleus formation was assessed. CHO-K1 cells were exposed, during targeted mitosis, to different concentrations of plant extract and cytochalasin B, in the presence and absence of an appropriate metabolic activation system (an S9 mix). The results obtained indicated that it might be possible to implement the Three Rs in assessing the potential human hazard of medicinal plants. The publication of such data can increase awareness of the Three Rs by showing how to optimise the management of animal use, if in vivo toxicological experiments are required.

Key words: CHO, cytotoxicity, Dipteryx alata, medicinal plant, micronucleus test, Three Rs.

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Introduction

Since the 1960s, the controversial issue concerning the use of laboratory animals in assessing the safety of products for human use has led to many studies involving alternative methods, i.e. those that use living cells and mammalian tissues instead of live animals (1). The Three Rs — Refinement, Reduction and Replacement — were originally described by Russell and Burch (2), and this was important to the scientific community, since the use of Three Rs alternative methods leads to a reduction in the number of animals used and, in some cases, to a complete replacement of the traditional in vivo tests.

According to our recent literature survey that used ‘medicinal plants’ and ‘human hazard’ as the key words, only 141 articles were found to have been published in the last 50 years. The same search, but restricted to papers published in 2009, found ten articles worldwide, but only three of these referred to alternative methods. The corresponding values for 2010 were eleven articles worldwide, with four about alternative methods. With regard to publications originating from Brazil, in 2009, a single paper was published with ‘medicinal plants’ and ‘alternative methods’ in its key words. The same key words did not retrieve any papers for 2010. However, searching with ‘medicinal plants’ and ‘cytotoxicity test’ as the key words, identified 13 Brazilian research papers that had been published during 2009 and 2010. In addition, in 2010 and from Brazil, only four articles focused on the use of the ‘micronucleus test’ to assess the genotoxicity of medicinal plants. In 2009, none had been published.

In this scenario, it is important to note the lack of specific international guidelines to investigate the potential cytotoxicity and genotoxicity of natural substances by using in vitro methods. In fact, the publication of such data might contribute to a