The safety of herbal medicinal products

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The primary aim of the EU, with the registration of herbal medicinal products (HMPs), is the protection of the European citizens from fraudulent and unsafe products. In fact, the EU is rather rigorous on this issue and therefore manufacturers are obliged to deal with safety issues. Although a Traditional Herbal Medicinal Products would have been in circulation for centuries, it is possible that with time, research proves the presence of toxic substances within the product. As herbal remedies are derived from nature, uneven conditions of growth and different varieties of a specific plant species may contribute to the emergence of previously-insignificant plant toxins. This has been also experienced with herbs and plants that are used for culinary purposes. Therefore to ensure the safety of herbal medicines, proof can be demonstrated by employing a battery of *in vitro* and *in vivo* tests.

The classical toxicity assays for herbal medicinal products are genotoxicity tests. These tests are based on the potential damage of plant constituents to DNA. The three most common forms of DNA damage and fixation are gene mutation (a change in the sequence of bases), chromosome mutation (structural alterations) and genome mutations (alterations in the chromosome number).

The front line genotoxicity test is the AMES[1] test. The test is performed by culturing *Salmonella typhimurium* or *Escherichia coli* strains that lack a specific amino acid, such as histidine or tryptophan, and challenging this culture with a suspected mutagen. A mammalian (rat) liver homogenate is added in case a compound requires metabolic activation prior to exhibiting its mutagenic effect. The revertent colonies are counted for the different mutagen concentrations to determine the extent of mutagenesis induced by the suspected compound.

The Mouse Lymphoma Assay is carried out on HMPs that exhibit a positive Ames test. Instead of the bacterial culture, this test utilises a mammalian cell model which distinguishes between gene and chromosome mutation. The result obtained is compared with a database containing information on different chemical entities.

The Rodent Micronucleus Test is appropriate to carry out if the DNA toxicant tested *in vitro* exhibits chromosomal alterations. In this test, the target organ is the bone marrow instead of the liver. Since the test involves the use of live animals, the rational use of animals, and whether the test is actually measuring what is expected, are taken into consideration. On the other hand, the use of an animal model may show more realistic results on the fate of the compounds under test. This establishes whether the compound requires hepatic activation, and whether it reaches the target organ.

Toxicological markers are widely used for herbal medicines, and these are classified into biological and chemical markers. Biological markers may be divided into direct and indirect toxicity indicators. The DNA-methyl green assay is an *in vitro* indirect method that determines the DNA binding capacity of potential toxicants. For potential toxicants which require metabolic activation, an indirect method involving the use of a rat model is utilised. The degree of toxicity can be determined by taking forestomach samples and separating the fragments by planar chromatography. This is typically performed for aristolochic[2] acid. Chemical markers are determined by analytical techniques such as GCMS for the presence of phellandrene in essential oils of plants, and HPLC analysis for the presence of coumarins in plant extracts.

More recently, *in silico* methods have been developed, superseding most *in vitro* and *in vivo* methods. Although *in silico* models are classically used to predict the binding capacity of substrates to receptors, these are now used to determine qualitative and quantitative structure-toxicity relationships (STRs). A STR is a qualitative model that associates the toxic properties with a chemical substructure (structural alert) or a property limit value. This is based on chemical similarities. Quantitative STR relates the structure of the potential toxicant to the toxicity of structures that have been already studied and included in a database. The prediction is not merely on a structural resemblance but on a value-based result with the degree of toxicity.

The technique of toxicogenomics is also a relative prediction tool. In this case, neither whole animals nor cell lines are used for testing. DNA microarrays reveal gene expression when a liver slice model is challenged with the potential toxicant. This data is compared to data of already-established toxicants in a database.[3]

Hepatotoxicity is one of the most important toxic effects, exhibited by substrates, on the human body. Risk assessment for hepatotoxicity is performed in an animal model. Doses are repeated at 28 days, 90 days and after 1 year and the effect is assessed as standard histopathology by light microscopy.[4] These tests are only mandatory for products authorized as medicinal products but not for those placed on the market as food supplements.

If toxicity is established for a marketed herbal medicinal product, this is immediately withdrawn from the market. However, if the product is presented for registration, it is not allowed to reach the market unless it meets the required safety standards. In the latter case, the product may be considered as a 'minus variant' which might require a slight modification. However, this modification should justify a safer profile.

References

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