Lack of genotoxicity of Rhamnus Purshiana, Sennae Fructus, and Rhamnus Frangula in a micronucleus assay in vitro (OECD 787)

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Antraquinones are substance contained in several species of Rheum palmatum, L., Rheum officinale Baillon, Cassia senna L., Rhamnus frangula L. and Rhamnus purshiana DC. These plants are commonly used as food supplements and in traditional medicine, mainly for their laxative properties. This study was conducted using dry extracts of three selected botanical species containing hydroxyantharacenes such as emodin and aloe-emodin, considered genotoxic by the EFSA-ANS Panel in 2018. This resulted in the EU decision to ban aloe-emodin and emodin in all extracts in which theses substance are present as the leaf of Aloe species but to temporary retain, under Community scrutiny, for two years, the use of extracts from the leaf, fruit of Cassia senna L., containing hydroxyanthracene derivatives, and extracts from the bark of Rhamnus frangula L., Rhamnus purshiana DC., containing hydroxyanthracene derivatives.

The aim of the present work was to evaluate the potential genotoxicity of botanical dry extracts obtained in accordance with European Pharmacopoeia of Rhamnus purshiana, Sennae fructus, and Rhamnus frangula, in a micronucleus assay in vitro (OECD 787). The micronucleus assay was performed on human lymphocytes obtained from the whole blood. All the treatments started after 48 hours of stimulation period with phytohaemagglutinin (PHA) when cells were actively proliferating. Two different treatments were performed: short treatment and continuous treatment. The first lasted for 3 hours, after when Cytochalasin B replaced the extract treatment; the second lasted 28 hours in co-treatment with Cytochalasin B. The short treatment was performed in presence and in absence of S9 metabolic activation. A very large range of concentrations was tested starting from 0 to 2000 µg/mL for Rhamnus purshiana, from 0 to 5000 µg/mL for Sennae fructus, and from 0to 2500 µg/mL for Rhamnus frangula. The continuous treatment was performed only in absence of S9 metabolism. The cytokinesis block proliferation index (CBPI) was then calculated as index of cytotoxicity.

The treatments do not induced cytotoxicity at any concentration. No statistically significant increase was observed for micronuclei formation in any of the tested doses and treatments. These results demonstrated that extracts of Rhamnus purshiana, Sennae fructus, and Rhamnus frangula extracts do not induce micronuclei in human lymphocytes after in vitro treatment at the tested concentrations.

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