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Protective role of St. John's Wort on formaldehyde-induced lung tissue injury: Inhibiton of inflammation and oxidative stress mediated apoptosis

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Formaldehyde (FA) is a ubiquitous environmental and occupational pollutant. It has been shown that its exposure is associated with inflammation and oxidative stress in the airways. St. John's Wort is a phytomedicine that has both anti-inflammatory and antiproliferative properties. The purpose of the present study was to investigate the protective effect of SJW against FA – induced lung toxicity and to evaluate the potential role of macrophage inflammatory protein 1 (MIP-1), TNF-alpha and, iNOS genes mediated oxidative stress and apoptosis.

A total of 32 Wistar albino rats were included and divided into 4 groups with 8 animals in each; Control, SJW, FA (6 ppm, 6 weeks by inhalation), FA+SJW groups. SJW was given at a dose of 300 mg/kg body. RT-PCR applied to detect the mRNA expression of MIP-1, TNF-alpha and, iNOS in the lung tissues. Caspase-3 levels were also measured with immunohistochemistry method.

According to RT-PCR analysis, the expressions of iNOS, MIP-1 and TNF-alpha were clearly enhanced by FA exposure ($p < 0.001$). The aggravation of damage in FA group was significantly prevented by SJW. Additionally, caspase-3 levels were significantly higher in the FA group compared to the other groups. These results suggest that increased inflammatory and apoptotic response to FA exposure is mediated by inflammatory genes and caspase-3. We suggested that SJW can be used as a promising protective agent against formaldehyde toxicity because of the obvious beneficial effects on inflammatory and apoptotic parameters.

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Alternative approaches for acute inhalation toxicity testing to address global regulatory and non-regulatory data requirements

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Inhalation is a major route of exposure to airborne contaminants. Therefore, understanding the hazards associated with inhaled materials such as environmental chemicals, household products, tobacco-based products, and other substances is vital. Acute systemic toxicity tests identify chemicals that could cause illness or death immediately or shortly after a single exposure. Regulatory testing for acute inhalation toxicity is often conducted following test guidelines from the Organisation for Economic Co-operation and Development (OECD). These tests are primarily based on lethality in rodents and provide little or no elucidation of the mechanisms of observed toxicity. To identify approaches that can reduce and replace animal use for acute inhalation toxicity testing, an international group of experts convened at a 2016 workshop co-hosted by the PETA International Science Consortium Ltd. and the US NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). The outcome of the workshop was the formation of working groups focused on: (1) developing a database of existing acute systemic toxicity data; (2) preparing a state-of-the-science review on mechanisms and assays for acute inhalation toxicity; (3) developing *in silico* models; and (4) conducting a proof-of-concept study to optimize an integrated approach comprised of *in vitro* and *in silico* methods that results in standardized protocols that can be used across laboratories. The overall goal of this work is to propose a defined strategy based on non-animal methods that can replace acute inhalation testing in animals for both regulatory and non-regulatory purposes.

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PLATOX – In vitro and in vivo investigations (28-day inhalation) to generate valid toxicity data for risk assessment of carbon-based nanoplatelets

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Carbon-based nanoplatelets (CNP) represent a new class of 2-D nanostructures in multiple variants and with interesting functional properties (material enforcement and electrical conductivity). A very high toxicity is not expected for nanoplatelets, however, hazard characterisation is still incomplete. Typical CNP candidates were selected, covering single layer/multilayer graphene, carboxyl graphene, single layer graphene oxide, and graphite oxide. Printex 90® served as particulate, non-platelet reference. The commercially available CNP (ACS Material, USA) were first characterised regarding sterility/endotoxin and morphology (SEM pictures) and the BET surface was re-evaluated. As *in vitro* screening models both, primary rat alveolar macrophages (AM) and MRC-5 human lung fibroblast cells were analysed on membrane damage (LDH release) and metabolic activity (AlamarBlue® test). Interestingly, the two single layer graphens induced marked concentration-dependent membrane damage in AM after 24 h of incubation, with a BMD30 of 3.2 and 2.5 $\mu g/cm^2$, whereas no such effect was observed for MRC-5 cells. Some LDH release was also observed for single layer graphite oxide (BMD30: 39.3 $\mu g/cm^2$). The other materials were nearly inactive. Significant effects on metabolic activity were not observed. In AM, single layer graphenes additionally induced direct DNA damage and release of PGE_2. In conclusion, single layer graphenes seem to possess (geno)toxic potential *in vitro* in AM, but not in lung fibroblasts. Based on the in *vitro* screening and for validation, two CNP were selected for a 4-week