E-CIGARETTES INDUCE *IN VIVO* TOXICOLOGICAL EFFECTS THAT CAN RAISE THE CANCER RISK

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Introduction: Electronic cigarettes (e-cigs) are devices thought to deliver nicotine in a vaping solution without tobacco combustion. Perceived as a safer alternative to conventional cigarettes, e-cigs are aggressively marketed as lifestyle-choice consumables, thanks to few regulatory restrictions. E-cigs have also gained popularity among never-smokers and teenagers, becoming an emergent public health issue. Despite the burgeoning worldwide consumption, their safety remains largely unproven and it is unknown whether these devices could cause *in vivo* toxicological effects that may contribute to cancer. The aim of the present study was to investigate several toxicological aspects associated with e-cig use including their mutagenic and co-mutagenic potential in a rat model.

Materials and methods: Male Sprague Dawley rats were randomly split into two groups: non-exposed and whole-body mode exposed (10 animals per group). Treated animals consumed 1mL/day of e-liquid containing 18mg/mL of nicotine. During the experiment the e-cigarette voltage was set at 5.5(and the wattage was about 15). Animals were exposed for 4hours/day for 5consecutive days/week, and for 4consecutive weeks.

Results: Toxic aldehydes production, due to thermal decomposition of glycerol, were detected in e-cig aerosol. In the exposed lung, we observed a significant increase in phase I CYP1A1/2, CYP2B1/2, CY2C11and CYP3A enzymes. The overproduction of reactive oxygen species resulting from CYP induction, together with the free radicals present in the aerosol, was confirmed by means "Electron Paramagnetic Resonance-radical probe" technique. Notably, we observed that the lung antioxidant enzymes catalase, DT-diaphorase, superoxide dismutase and the conjugating phase II glutathione S-transferases were all markedly reduced in e-cig-exposed animals. In addition, we found a markedly reduced FRAP (ferric reducing antioxidant power) value in the lung. Moreover, plasma FRAP levels and measurement of carbonyl residues, as biomarkers of oxidative injury to proteins, were inversely correlated in e-cig vapour-treated rats. We also found that 8-hydro-2′-deoxyguanosine raised in the lungs of exposed animals. Interestingly, e-cigs vapour produces extensive DNA damage in leukocytes (Comet test) and determines an increase in the percentage of immature micronucleated reticulocytes over normal reticulocytes. Next, the urine of e-cig-exposed animals induced a dose-dependent increase in the number of S. typhimurium revertants in different strains.

Discussion and conclusions: We found that e-cigs determines a powerful booster effect on phase-I carcinogen-bioactivating enzymes, and an increased oxygen free radical production and DNA oxidation to 8-hydroxy- 2′-deoxyguanosine. Furthermore, we found that e-cigs damage DNA not only at chromosomal level in peripheral blood, such as strand breaks in leucocytes and micronuclei formation in reticulocytes, but also at gene level such as point mutations in urine. As these detrimental phenomena are typically induced by conventional cigarettes, the erroneous belief that e-cigs are safe should be retracted and suitable measures implemented to protect public health. Our study should be seen as the starting point for further investigations designed to confirm the harmful health impact of e-cigs, particularly under different usage conditions and after long-term exposure.