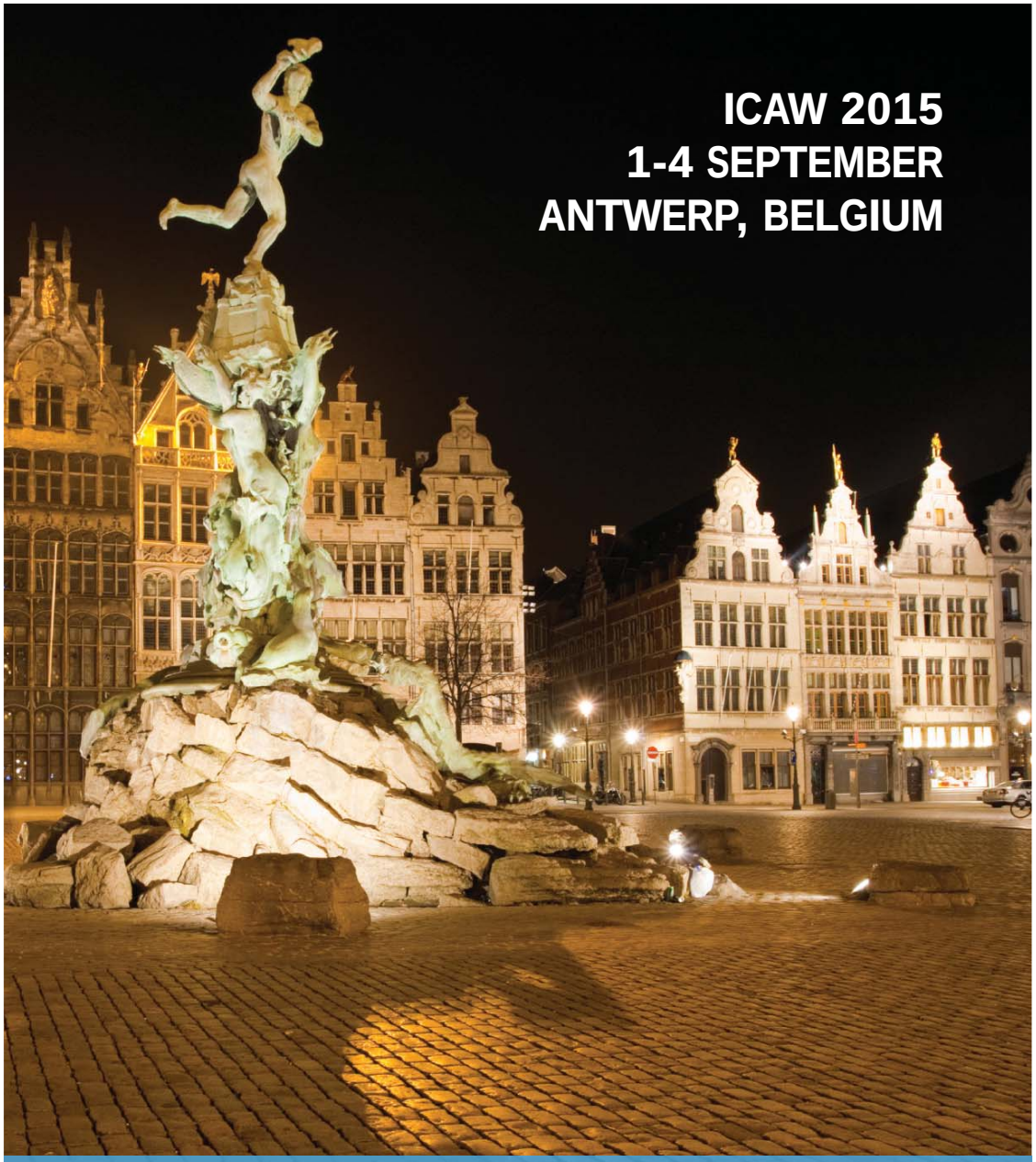

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P-6: Protective effect of Crataegus monogyna Jacq. ethanolic extracts in oxidant-induced DNA damage evaluated through comet assay with human peripheral lymphocytes

Joao C. Barreira[1, 2], Carla Costa[3, 4], João P. Teixeira[3, 4], Isabel C. Ferreira[2] and Beatriz P. Oliveira[1]*
[1]Faculty of Pharmacy - University of Porto, Portugal
[2]Polytechnic Institute of Bragança, Portugal
[3]Department of Environmental Health, Portuguese National Institute of Health, Portugal
[4]EPIUnit - Institute of Public Health, Portugal

Much attention of preventive medicine research is focused on natural antioxidants. This interest refers not only to isolation and identification of new biologically active molecules for the pharmaceutical industry, but also because of the emergent public interest in using crude plant extracts, such as infusions for self-medication (Krishnaiah et al., 2011). The use of antioxidants, such as the well-known polyphenolic compounds, to prevent genetic damage induced by physical or chemical agents is of considerable interest. This bioactivity might be related to their anticlastogenic effect, due to the presence of specific functional groups. Other antioxidant compounds, such as vitamins C and D, were reported for their DNA-damage decreasing effect, suggesting that reactive oxygen species may be involved in this activity (Benavente-García et al., 2004). Evaluating the antioxidant activity of natural matrices represents one of our primary research challenges (Ferreira et al., 2009). Among hundreds of studied species, Crataegus monogyna Jacq. stood out as being one of the most promising plants due to its high bioactivity. Besides the antioxidant activity, C. monogyna was also studied for the human tumour cells growth inhibitory capacity of its phenolic extracts; furthermore, individual phenolic compounds were fully characterized by high performance liquid chromatography-photo diode array detection-electrospray ionization tandem mass spectrometry (HPLC-DAD-ESI/MS), revealing high levels of flavonols, flavones, hydroxycinnamic acid derivatives and anthocyanins (Rodrigues et al., 2012). However, there is a high limitation in examining if the detected bioactivity is actually transferred from in vitro to in vivo systems (Carocho and Ferreira, 2013). Nevertheless, there have been major advances to accurately measure end products of oxidative damage to proteins, lipids, and DNA. Short-term in vitro tests are commonly used to identify genotoxicants and antigenotoxicants. Comet assay, in particular, has been considered to be a useful biomarker to this purpose as it can easily establish a link between natural extracts and DNA repair damage (Cemeli et al., 2009). Although this assay might be carried out virtually with any cell type, lymphocytes are often used as these are in contact with xenobiotics after absorption and have proved to be good surrogate cells (Cemeli et al., 2009). Herein, the potential genotoxic and antigenotoxic effect of ethanolic extracts from different botanical parts of C. monogyna was evaluated by the alkaline comet assay. Extracts were assayed to match the previously determined effective concentrations (EC) corresponding to 25% (EC_{25}), 37.5% ($EC_{37.5}$), 50% (EC_{50}), 62.5% ($EC_{62.5}$) and 75% (EC_{75}) of antioxidant activity. Blood samples were obtained from healthy non-smoking donors in EDTA tubes and lymphocytes were isolated by Ficoll density gradient. A pool of lymphocytes was exposed to extract in different concentrations in addition to a positive and negative control for 1h to test

for extracts' genotoxicity. To test for extracts antigenotoxicity, two concentrations were selected and cells were exposed to a mutagenic compound (MMS) and extract concentrations simultaneously for 1 h. In one experiment, comet assay was immediately performed after the exposure period while in the second experiment, cell media were renewed and the comet assay was performed after a recovery-period of 1 h. All experiments were carried out with and without metabolic activation by liver fraction (S9). The results showed significant differences within the assessed botanical parts and also among the assayed concentrations. The performed investigation might be considered as representing a step further in the evaluation of the in vivo bioactive potential of this highly promising species. Furthermore, it has established some practical bases for the evaluation of additional natural matrices with high scoring in bioactivity screening studies.

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