

gratefully acknowledges Junta de Andalucía for her predoctoral grant associated to the AGR7252 project.

<http://dx.doi.org/10.1016/j.toxlet.2015.08.243>

P02-018

Use of single cell gel electrophoresis assessing DNA damage caused by chemopreventive agents in human leukaemia cells. A model for nutraceutical in vitro screening



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Cancer is one of the leading causes of death world-wide. One of its major pathological pathways is the silencing of the apoptosis mechanism, producing uncontrolled cellular growth. Therefore, agents that can trigger apoptosis in tumour cells can be potentially considered as chemopreventives. In this study we determined the ability to induce DNA single/double-strand breaks, a pro-apoptotic major marker, of several nutritional compounds using the Single Cell Gel Electrophoresis (SCGE – comet assay) and the internucleosomic fragmentation assay (IF), as well their cytotoxic activity in order to compare the sensibility of both methodologies as nutraceutical screening tools. In total, six worldwide highly consumed nutritional products (two beverages: ale stout beer and cola drink; two food additives: glutamate and citric acid; and two vegetables: garlic and onion) were assessed to ascertain on their clastogenic activity against a HL-60 line. Cells in the exponential growth phase (10^6 cells/mL) were treated during 5 h with three different concentrations (low, medium and high) of the selected substances and, after alkaline lysis and electrophoresis, DNA of single cells were visualised fluorescent microscopy using PI dye. Our results showed that ale stout beer was cytotoxic and significantly increase both proapoptotic internucleosomic DNA fragmentation and comet tail area at medium concentrations. The same pattern was observed in garlic extract (also at medium concentration) but IF was weaker than before. Cola drink was also cytotoxic, weakly activated DNA fragmentation at very high concentrations and significantly increased comet tail area only at high. Finally, citric acid, glutamate and onion extract treatments did not produced differences with the control cells in all the assessed parameters (IF, SCGE and cytotoxicity). In conclusion, cytotoxic, DNA internucleosomic fragmentation and comet data were congruent and supplementary. The heterogeneity of DNA damage in explaining resistance to cancer treatment can be used in cell from tumour models for food safety assessment.

<http://dx.doi.org/10.1016/j.toxlet.2015.08.244>

P02-019

Toxicological, genotoxicological, cytotoxic and lifespan induced activities of different types of garlic (white, purple and black)



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Allium sativum has been used throughout history for medicinal purposes and takes part in the healthy components used in the Mediterranean diet as well. There is a wide range of researches

which are focused on the health promoting properties of garlic. White, purple and three types of black garlic (OC1, 1C2 and 2C1, with different fermenting times) were selected in order to check some possible differences in their nutraceutical potential. For this purpose, *in vivo* (toxi/antitoxicity, geno/antigenotoxicity and longevity assays in *Drosophila melanogaster* model) and *in vitro* (cytotoxicity and proapoptotic DNA internucleosomic fragmentation in HL-60 human leukaemia cell line) assays were carried out. These studies have shown that all types of garlic were safe without showing toxicity, although they did not exert protective effects against the oxidative agent hydrogen peroxide, with the exception of OC1 black garlic that showed a slight protection against the oxidative toxicant. With respect to the genotoxic potential, all raw and processed garlic material were not genotoxic with the exception of the higher concentration of white garlic. On the other hand, the highest concentrations of white and 2C1 garlic and the lowest concentration of OC1 and purple garlic did not exhibit antigenotoxic effects when the imaginal discs were treated with the genotoxic hydrogen peroxide. The longevity assays in *Drosophila* yielded significant extension of lifespan results only in some of the tested concentrations of white and OC1 and 1C2 black garlic; and contrarily, purple garlic decreased the lifespan extension at the higher concentration. Finally, the results achieved in the *in vitro* experiments for garlic cytotoxicity were hopeful. All studied garlic induced a decrease in leukaemia cells growth, being the purple and white ones the healthiest with an IC_{50} lower than 0.003 mg/ml and 0.02 mg/ml respectively, whereas the other sorts of garlic had an IC_{50} near 1 mg/ml concentration. However, the purple garlic breed was the only inductor of cellular death by the DNA proapoptotic way. In conclusion, our data suggest that garlic could be proposed as a nutraceutical substance but a concentration effect is observed in all the assays being the higher doses not advisable.

<http://dx.doi.org/10.1016/j.toxlet.2015.08.245>

P02-020

The toxic effects of Cacti-Nea™ on germination of *A. cepa*



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Opuntia ficus indica L., a species in the cactus family Cactaceae, it is a plant growing in dry and hot climates: northern Mexico, south-western United States, Africa, Mediterranean countries and Europe. The fruits are used in the traditional medicine. NEXIRA Health develops Cacti-Nea™, a cactus fruit extract with natural diuretic properties, it is a dehydrated water extract of the fruits of the prickly pear cactus *Opuntia ficus indica*, obtained by a process designed to preserve the nutritional and functional properties of the fruit. Nutraceutical can be defined as, “a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease”. However, the term nutraceutical, as commonly used, in marketing has no regulatory definition. Nutraceuticals and food supplements frequently contain huge dosages of antioxidants. It is not generally recognized that high intake of antioxidants may also have adverse effects. This study proposes the evaluation of Cacti-Nea on

germinations of *Allium cepa* and evaluation of antioxidant activity of Cacti-Nea through the DPPH. Where used eleven concentrations for the evaluation of the toxicity. The nutraceutical Cacti-Nea (20 mg/mL) showed antioxidant activity of 81.21 ± 0.004243 , this result appointed the relevance of this nutraceutical, however three concentrations (1 g/mL, 0.5 g/mL and 0.12 g/mL) of Cacti Nea showed inhibition of the germination *A. cepa*. Concentrations of 0.006, 0.004 and 0.002 g/mL has the satisfactory germination rate of 83%. This result indicates possible correlation between dose and effect. These results demonstrate the necessity for toxicological tests in nutraceuticals, other tests will be performed to evaluate other acute and chronic toxicological parameters.

<http://dx.doi.org/10.1016/j.toxlet.2015.08.246>

P02-022

In vivo genotoxicity evaluation by standard and modified comet assay in PTSO (propyl thiosulphinate oxide) a garlic oil compound



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Essential oils (Eos) are a good source of several bioactive compounds, which possess antioxidative and antimicrobial properties. In addition, some EOs have been use in food industry and can be incorporated into packaging in which they can provide multifunctions termed “active or smart packaging.” The aim of this study was assess the possible genotoxic effects of PTSO (propyl propane thiosulfate), an oil sulfured compound from *Allium* species, developed and isolated chemically by DOMCA (Granada, Spain). For this reason, genotoxicity was determined in Wistar rats by the standard and modified comet assay according to the principles of OECD guideline 487. The animals were administrated by gavage with PTSO 5.5, 17.39 and 55 mg/kg b.w and treated at three intervals of 0, 24 and 45 h. 3 h after the last dose they were sacrificed and the liver and stomach were obtained. The standard comet assay was performed to detect DNA strand breaks. In addition, possible oxidative DNA damage was measured adding restrictions enzymes to the isolated nucleus. In this sense, formamidopyrimidine DNA glycosylase (FPG) and endonuclease III (Endo III), allow the measurement of oxidised pyrimidines and purines bases, respectively. Preliminary results obtained for the standard comet assay indicate no significant increase DNA strand breakage in any concentration tested. However, it is necessary to evaluate the results in the FPG-End III-modified comet assay to explore the safety of this compound and its future use in food active packaging.

Acknowledgements: The authors wish to thank the Spanish Ministry of Science and Innovation (AGL2012-38357-C02-01) co-financed by FEDER Funds, and Junta de Andalucía (AGR-7252) for the financial support for this study; and the Cell Culture Service and Microscopy Service of CITIUS from the University of Seville for technical support.

<http://dx.doi.org/10.1016/j.toxlet.2015.08.247>

P02-023

Reduction of arsenic bioavailability using dietary strategies



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The main route of exposure to arsenic (As) is the consumption of water and certain foods, where the forms with greater toxicity are inorganic [As(V), As(III)] and dimethylarsinic acid, DMA(V). It has been shown that the chemical form of As influences its intestinal absorption, and therefore the amount of element that reaches the systemic circulation and exerts its toxic effect. Some studies show that inorganic As is fully absorbed while the DMA(V) has a lower degree of absorption. It has also been reported that presence of food reduces the bioaccessibility (fraction solubilized during gastrointestinal digestion). It is therefore plausible that some food components diminish the As absorption and therefore its toxicity. The objective of this study is the search for food components or dietary supplements that reduce the bioaccessibility of As(III), As(V) and DMA(V) from food, in order to reduce the amount of As available for absorption. For this purpose, 37 components or dietary supplements have been assayed using a static *in vitro* model of gastrointestinal digestion. Initially we have evaluated the effect of the compounds on the solubility of aqueous standards solutions of arsenic species submitted to the conditions of the gastrointestinal digestion. Those components that have reduced the solubility of aqueous standards have been tested in three food matrices (white and brown rice and seaweed) to determine the effect of their presence in the bioaccessibility of As. The results show that sulfates of Fe(II) and Fe(III), Ca with phytates, certain types of cellulose, xylans, N-acetylcysteine and saponins are those compounds which reduce further the solubility of the three forms of As in aqueous solution. We must highlight the significant reduction observed with Fe, which annuls the solubility of inorganic forms and reduces more than 50% that of DMA(V). This significant reduction is also observed in rice (100%) and seaweed (60%). The remaining components are not as effective in food, possibly due to their interaction with other matrix components that hinder the binding of arsenic forms. The use of dietary supplements of Fe confirms the capacity of Fe to reduce the bioaccessibility of As, showing that the use of Fe may be a good strategy to reduce the entrance of As into the systemic circulation.

<http://dx.doi.org/10.1016/j.toxlet.2015.08.248>

P02-024

Hydroxyphosphonoacetic acid – An accidental contamination in drinking water



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Hydroxyphosphonoacetic acid (HPAA) is used in industrial water supplies to prevent corrosion of steel tubes; for corrosion inhibition a 1:1-mixture of HPAA with polyacrylic acid (PAA) is added to high mineral water at a concentration of 1:10,000. It is not intended to be used in drinking water and has not been evaluated toxicologically. In a large office building the industrial mixture