Introduction

Genotoxicity is a term in genetics defined as a destructive effect on the genetic material of a cell (DNA, RNA) that affects its integrity (Cinthura. Gayathri, & Priya, 2017). The genotoxic potential of many chemical compounds depends on the biological system and the concentrations used. Animal models are still required for more complex evaluation of the chemical and biological properties of the different compounds found in plants, for which it is necessary to assess the maximum limit of pain, suffering and anguish to which animals can be exposed submitted.

In ovo tests are commonly known for their potential to replace animal tests in preclinical trials. In ovo tests provide the possibility of evaluating a large number of samples in a non-invasive, fast, simple, reliable and cost-effective way. Quercetin is considered the main flavonoid in the human diet (Iacopetta et al., 2017). Despite the several benefits of quercetin to vertebrates, quercetin has a chemical structure susceptible to the formation of compounds capable of creating adducts with DNA, compromising the genetic stability of the cell (Cavallieri, Rogan, & Chakravarti, 2004).

Aim

➢ Adapt the in ovo protocol to the micronucleus testing of phytochemicals;
➢ evaluate the genotoxic potential of quercetin using the in ovo model – Hen’s egg test – micronucleus (HET-MN) test;

Methodology

Fertilized eggs from Pintobar - Poultry Farm, Lda (Amares, Portugal) were incubated for 11 days (d0 - d11, with d1 corresponding to the first 24 hours) at 37.5 - 38 °C temperature with a relative humidity of 70 - 80%. Three experimental groups of embryos exposed to quercetin, corresponding to doses 1 µg, 10 µg and 100 µg of quercetin per embryo (66.7 µg / ml, diluted in DMSO and PBS), being inoculated on the eighth day of incubation, 150 µl / egg.

On d11 (72 hours after exposure to quercetin), an incision was made such as to place it in the largest vessel accessible (umbilical or chorioallantoic vessels), and through it 200 µl of blood was extracted. The micronuclear count in blood smears was carried out manually and single blind (sample identity not disclosed to scorer). Statistical analysis was performed using the statistical program Graphpad Prinml (San Diego, CA-USA). The results from control vs. experimental groups were compared by univariate analysis (one way analysis of variance / ANOVA) with Hotm-Sidak post-hoc test. For all comparisons, statistically significant differences were considered for the significance levels of P <0.05, <0.01 and <0.001.

Results

The data presented reflect the result of three tests performed each with an average of five (5) eggs / test for each experimental group, whose objective was to assess the genotoxic potential of quercetin, having as a marker the number the micronuclei formed in the erythrocytes of the extracted blood.

As initial morphologic test, it was confirmed that, compared to other blood cells, erythrocytes represented greater globular volume, typically having an oval or elliptical shape, with an oval nucleus, usually located in the center of the cytoplasm; thrombocytes showed a similar shape to erythrocytes (oval), however, they were smaller and contained granules that occupied a large part of their total volume, while leucocytes were polymorphic (Figure 1).

Micronuclei were detected in different types of erythrocyte (Figure 2)

Embryo viability was not significantly different between conditions. However, viability was reduced to 40 % in the MMS mutagenic control (Table 1). Dose-dependent induction of micronucleus formation was observed after the administration of quercetin (Figure 3).

Although micronucleus induction was observed in all experimental groups, groups II and III (1 and 10 µg quercetin) did not present statistically significant differences when compared to each other and to group I, solvent control (1% DMSO in PBS). Conversely, in group IV (100 µg quercetin), an increase was observed in micronucleated erythrocytes compared to solvent control.

Conclusion

In HET-MN, there was an increase in the frequency of micronuclei in the groups treated with quercetin, this increase being proportional to the concentration of quercetin administered. These results suggest a genotoxic effect of quercetin in the embryos of G. gallus domesticus, which is hypothesized to be related to self-oxidation of quercetin, and reactivity of the catechol group.

References


Acknowledgments

The authors acknowledge funding from FCT/Fundaçao para a Ciência e a Tecnologia and MCTES through national funds and Programa Operacional Competitividade e Internacionalização (COMPETE), grant number POCI/MED-MD/02834/2017; POCI/01/0145-FEDER/02834 and from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through grant UID05/0056/2020. LMAFJ.Д. (SFRH/BPD/74666/2010) thanks FCT for funding through program D. 57/2016 – Norma transitória.