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# Evaluation of mutagenic activity of platinum complexes in somatic cells of *Drosophila melanogaster*

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ABSTRACT

Cisplatin, carboplatin, and oxaliplatin are some of the most often used alkylating chemotherapeutic agents. In view of the paucity of data on the genotoxicity of oxaliplatin, this study compares the mutagenic activity of cisplatin (0.006, 0.012, 0.025, 0.05 mM), carboplatin (0.1, 0.2, 0,5, 1.0 mM), and oxaliplatin (0.1, 0.2, 0,5, 1.0 mM) using the somatic mutation and recombination test (SMART) in *Drosophila melanogaster*. Standard and high-bioactivation crosses of the drosophilid were used, which present basal and high levels of cytochrome P450 (CYP450) metabolization enzymes, respectively. All concentrations of cisplatin and carboplatin induced lesions in genetic material in both crosses, while oxaliplatin was mutagenic only to high bioactivation flies treated with 0.1, 0.5 and 1 mM of the compound. No significant differences were observed between genotoxicity values of cisplatin and carboplatin. However, CYP450 enzymes may have affected the mutagenic action of oxaliplatin. Carboplatin induced mainly mutation events, while cisplatin triggered mostly mutation and recombination events when low and high doses were used. Most events induced by oxaliplatin were generated by somatic recombination. Important differences were observed in genotoxic potential of platinum chemotherapeutic compounds, possibly due to the origin and type of the lesions induced in DNA and the repair mechanisms involved.

## 1. Introduction

Whether they are used individually or in combinations, platinum chemotherapeutic agents remain some of the most effective alternatives in cancer treatment approximately five decades after they were first discovered (Misiak et al., 2016). With the introduction of cisplatin other platinum complexes like oxaliplatin and carboplatin (Fig. 1) have been developed, exhibiting promising anticancer properties and earning an important position in international pharmaceutics markets (Spreckelmeyer et al., 2014). More recently other platinum chemotherapeutic compounds have been launched, like nedaplatin, lobaplatin, and heptaplatin, which are traded only in Japan, China, and Korea, respectively (Jung and Lippard, 2007; Wheate et al., 2010; Ali et al., 2013). Research has shown that oxaliplatin offers additional advantages compared with cisplatin, such as lower resistance of tumor cells to chemotherapy and fewer side effects (Ali et al., 2013).

However, despite widespread use secondary toxic effects such as mutagenicity have been reported in healthy cells both *in vivo* and *in vitro* due to the non-selective action of platinum chemotherapeutic agents, increasing the risk of secondary tumors (Wu et al., 2011; Antunes et al., 2005; Khynriam and Prasad, 2003; Kosminder et al., 2004; Oliveira et al., 2009; Rjiba-Touati et al., 2012; Serpeloni et al., 2013; Katz, 1987; Danesi et al., 2010a,b; Quita et al., 2012; Souza et al., 2017; De Souza et al., 2017). The action mechanism of these platinum complexes is based on interchain crosslinking or interchain DNA bridging between two adjacent guanine molecules or between guanine and adenine molecules. The main effect is the inhibition of DNA replication (Francesco et al., 2002; Deepa et al., 2013). In addition, the direct and indirect interactions with proteins, RNA, and enzymes increases the complexity of apoptosis, which plays a central role in antitumor effect (Almeida et al., 2006; Hostetter et al., 2009; Ndinguri et al., 2009).

In view of the paucity of information about the mutagenic action of oxaliplatin and the absence of data on the recombinagenic action of platinum complexes, the present study assessed the genotoxicity of the chemotherapeutic agents cisplatin, carboplatin, and oxaliplatin using the somatic mutation and recombination test (SMART) in *Drosophila*

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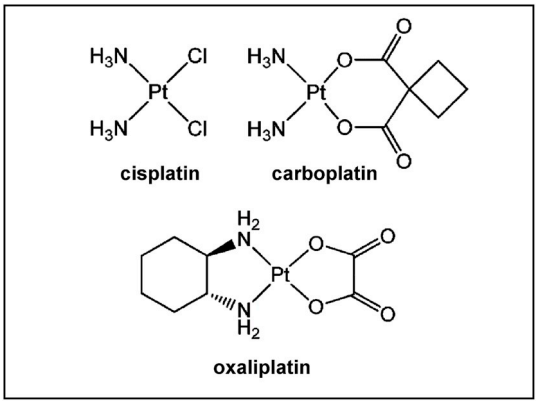


**Fig. 1.** Chemical structure of platinum complexes cisplatin, carboplatin, and oxaliplatin. Modified from Ang et al., 2010).

*melanogaster*.

## 2. Materials and methods

### 2.1. Chemicals

Cisplatin, carboplatin, and oxaliplatin were used as the pharmacological drugs available in the market (Citoplax®, Tecnocarb®, and Lipoxal®, produced by Laboratório Químico Farmacêutico Bergamo Ltda., Taboão da Serra, Brazil; Zodiac Produtos Farmacêuticos S.A., São Paulo, Brazil; and Laboratório IMA S.A.I.C., Buenos Aires, Argentina, respectively).

Urethane 20 mM (CAS 51-79-6, Sigma-Aldrich, St. Louis, MO, USA) was used as positive control.

All solutions used were prepared immediately before use in distilled water.

### 2.2. Drosophila tester strains and collection of larvae for treatment

The SMART is carried out using *D. melanogaster* strains carrying specific marker genes located in chromosome 3, allowing the detection of mutation and recombination of somatic cells of the fly species. These strains are called *flr*$^3$ (*flr*$^3$/*In*(3LR)*TM3, ri p*$^p$ *sep l(3)89Aa bx*$^{34e}$ *e Bd*$^S$), ORR; *flr*$^3$(*ORR/ORR; flr*$^3$/*In*(3LR)*TM3, ri p*$^p$ *sep l(3)89Aa bx*$^{34e}$ *e Bd*$^S$) and *mwh* (*mwh/mwh*). Standard cross (ST), in which virgin *flr*$^3$ females are crossed with *mwh* males, generating larvae with a basal P450 metabolic activity (Graf et al., 1989) and the high bioactivation cross (HB), which is based on the crossing of virgin ORR;*flr*$^3$ females carrying chromosomes 1 and 2 from the Oregon (R) strain resistant to dichlorodiphenyl-trichloroethane (DDT), were used (Frölich and Würgler, 1989). A principal gene (R) present in chromosome 2, together with other genes in chromosome 1, is responsible for a high level of cytochrome P450 enzymes (Graf and van Schaik, 1992). Information about marker genes is available in Lindsley and Zimm (1992).

All *D. melanogaster* stocks and crossings were kept in 250-mL vials containing standard growth medium at 25 °C ± 1 °C and relative humidity between 60% and 70%.

Crossings were carried out by mass breeding (80 females to 40 males) for 3 days in vials containing standard growth medium. Then, flies were transferred to 250 mL vials containing oviposition medium (sugar, water, and baker's yeast), where they remained for 8 h. Third-instar larvae were collected 72 h after the beginning of oviposition by floatation in distilled water and transferred to plastic tubes containing 1.5 g *Drosophila* instant medium (Carolina Biological Supply, Burlington, NC, USA).

Treatments were started adding 5 mL of each test solution of cisplatin, oxaliplatin, and carboplatin. The concentrations used (cisplatin: 0.006, 0.012, 0.025, 0.05 mM; carboplatin: 0.1, 0.2, 0,5, 1.0 mM; oxaliplatin: 0.1, 0.2, 0,5, 1.0 mM) were defined based on a toxicity test performed with 100 larvae treated with sequential concentrations of each platinum complex considering a minimum survival rate of 70%.

After hatching, flies from the two genotypes (trans-heterozygous for marker genes *mhw* and *flr*$^3$, *mhw/flr*$^3$, and heterozygous for chromosome *TM3*, *mhw/TM3*), wings were detached and mounted on slides and analyzed in an optical microscope (400x). During larva development, changes in gene material of cells of the imaginal disk induces the formation of spots on adult flies' wings as multiple spots or large spots, which reflect the recessive genes *mwh* and/or *flr*$^3$, respectively (Graf et al., 1989).

The different types of spots are called single *mwh* or *flr*$^3$, when only one marker is expressed, or twin spots, when both mutant genotypes (multiple wing hair, *mwh*) and flare (*flr*$^3$) are present in the same spot. Single spots with two or one mutant cells are called small; the others are called large (Graf et al., 1984).

The type of spot visualized on the wings of trans-heterozygous adult flies allows characterizing distinct genotoxicity events. Single spots may originate from mutation, chromosome aberration, or somatic recombination. In turn, twin spots, which express the phenotypes *flr*$^3$ and *mwh* concomitantly result exclusively from recombinational events, which involve the occurrence of one single recombination between *flr*$^3$ and the centromere, when a recombinant chromosome and a parental chromosome are subsequently segregated (Graf et al., 1989).

In *mwh/TM3* flies, recombination between the balancer chromosome *TM3* and its structurally normal homologue is a lethal event. The comparison between the number of spots obtained in both genotypes enabled quantifying the recombination and mutation events (Graf et al., 1989). Clone induction frequency per $10^5$ cells per cell division were also calculated based on the number of *mwh* clones obtained in the different treatments. Initially, it is possible to estimate the damage induction frequency per cell and cell division dividing the number of *mwh* (*n*) spots by the result of the multiplication of the total number of cells analyzed in both wings of a fly ($C$ = 48,800 cells) by total number of flies analyzed ($N$) using the equation $n/N$x48,000. Subsequently, these values were multiplied by 100,000 to express frequency in $10^5$ cells (Frei and Würgler, 1988). This calculation was carried out using only *mwh* spots due to the poor expression of the *flr*$^3$ marker in small spots and to the lethality of this marker in large spots (Frei et al., 1992).

Based on clone induction frequency values per $10^5$ cells per cell division, the percent values of genetic damage generated by somatic recombination (R) and mutation (M) induced by the studied drugs were calculated using the equations described by Frei and Würgler (1996): (i) R = 1 [(Frequency of clone formation per $10^5$ cells in *mwh/TM3*)/(Frequency of clone formation per $10^5$ cells in *mwh/flr*$^3$ flies)] x 100 and (ii) M = 100 − R. These calculations were conducted using the spot frequency values corrected by the negative control.

### 2.3. Statistical analyses

Spot frequency after treatments with different concentrations of cisplatin, oxaliplatin, and carboplatin were compared with the values obtained for the negative control using the conditional binomial test (Kastenbaum and Bowman, 1970) according to the multiple decision procedure developed by Frei and Würgler (1988).Frei and Würgler, 1995

## 3. Results

The present study evaluated the genotoxic potential of cisplatin, oxaliplatin, and carboplatin using the SMART in *D. melanogaster* wings. HB and ST crossings were used. Based on the analysis of toxicity test data shown in Figs. 2 and 3 all drugs were toxic and only the concentrations that effectively allow a survival higher than 70% in both crosses were applied to perform the genotoxic evaluation of these compounds. Although oxaliplatin had survival rates higher than 70% at

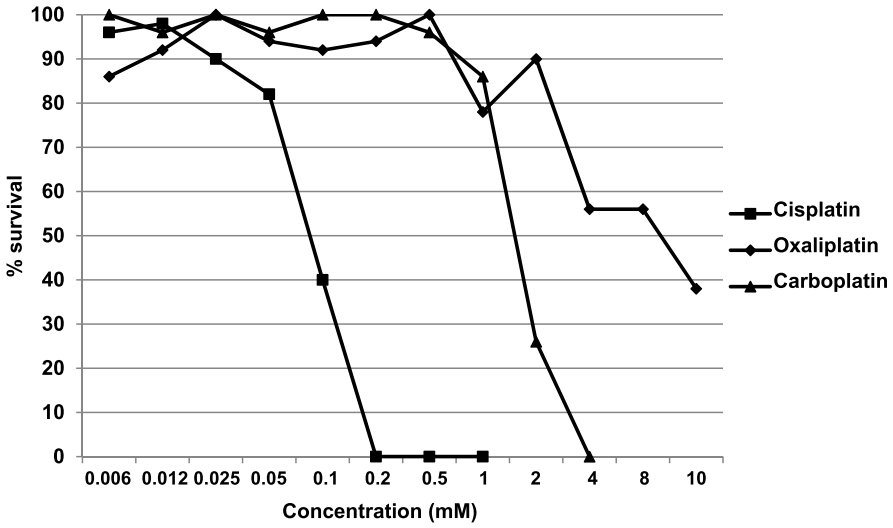


**Fig. 2.** Percent number of ST cross flies emerging from third-instar larvae that survived chronic exposure to different concentrations of cisplatin, carboplatin and oxaliplatin.

2 mM concentration, it was not used for genotoxicity assessment in order to maintain the same range of concentrations used for carboplatin (1–0.1 mM).

The results obtained for the ST cross show that concentrations of cisplatin and carboplatin (Table 1) increased the frequency of genetic damage in flies of both genotypes, in an evident dose-dependent relationship. The quantification of damage for origin shows that different concentrations of cisplatin induced distinct effects. Low concentrations (0.006 mM and 0.012 mM) caused preferably mutation events (88.65% and 51,83%, respectively). High concentrations (0.025 mM and 0.05 mM) induced higher percent values of recombination events (56.31% and 61.66%, respectively). On the other hand, all concentrations of carboplatin induced preferably genetic damage caused by mutation events (78.6%–85.16%). Oppositely to carboplatin and cisplatin, no concentration of oxaliplatin induced any increase in mutant spot frequency in the ST cross (Table 1).

Table 2 shows the results obtained for the HB cross. Overall, cisplatin and carboplatin induced similar results as observed for ST flies. Except for twin spots observed in genotype *mwh/flr*$^3$ after treatment with carboplatin, overall the treatment of both genotypes with the compounds induced a significant, dose-dependent increase in spot frequency values. This absence of increase in the frequency of twin spots, which are formed exclusively from recombination events, is similar to the data about the quantification of mutation and recombination events. Carboplatin induced mainly genetic damage caused by mutation events (between 73.98% and 82.94%). On the other hand, cisplatin, similarly to what was observed for the ST cross, induced preferably damage caused by mutation events when the low concentrations were used (68.67% and 58.65%) and damage caused by recombination events when the concentrations used were high (53.16% and 53.33%). Oxaliplatin presented genotoxic activity at the HB cross at concentrations of 0.1; 0.5 and 1 mM only in the *mwh/flr*$^3$ genotype, showing that this drug induced preferentially damage caused by recombination, ranging from 74.64 to 83.82%.

## 4. Discussion

Considering the wide use of platinum complexes in the treatment of several cancer types and the paucity of data on the genotoxicity of oxaliplatin, the present study assessed the mutagenic action of this

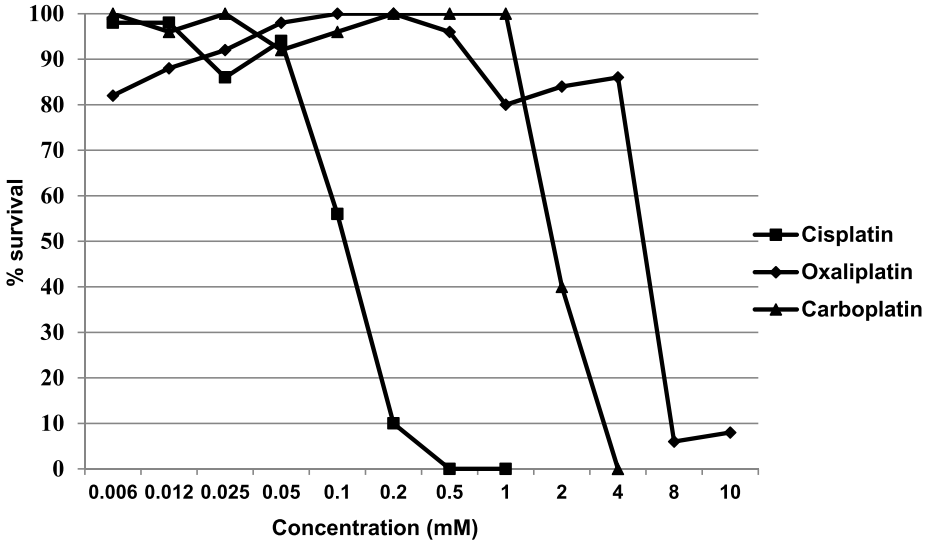


**Fig. 3.** Percent number of HB cross flies emerging from third-instar larvae that survived chronic exposure to different concentrations of cisplatin, carboplatin and oxaliplatin.

**Table 1**
SMART results in *mwh/flr*$^3$ and *mwh/TM3* progeny of the ST cross after the chronic exposure of larvae with cisplatin, carboplatin, and oxaliplatin.

| Treatments, drugs and genotypes[a] | Nº of flies (*N*) | Spots per fly (no. of spots) statistical diagnosis[b] | | | | | | | | | | | Spots with *mwh* | Frequency of clone formation per $10^5$ cells (*n/NC*)[f] | | Recombination[h] (%) | Mutation[h] (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Small single spots[c] (1–2 cells) *m = 2* | | | Large single spots[c] (> 2 cells) *m = 5* | | | Twin spots[d] *m = 5* | | | Total spots[e] *m = 2* | | | clone[e] (*n*) | Observed | Control Corrected[g] | |
| Negative control *mwh/flr*$^3$ | 50 | 1.30 | (65) | | 0.16 | (08) | | 0.08 | (04) | | 1.54 | (77) | | 76 | 3.11 | | |
| Negative control *mwh/TM3* | 40 | 1.08 | (43) | | 0.10 | (04) | | nc | | | 1.18 | (47) | | 47 | 2.41 | | |
| Positive control *mwh/flr*$^3$ | 20 | 10.05 | (201) | + | 1.95 | (39) | + | 0.40 | (08) | + | 12.40 | (248) | + | 244 | 25.00 | 16.11 | |
| **Cisplatin** | | | | | | | | | | | | | | | | | |
| *mwh/flr*$^3$ | | | | | | | | | | | | | | | | | |
| 0.006 mM | 50 | 3.00 | (150) | + | 1.04 | (52) | + | 0.36 | (18) | + | 4.40 | (220) | + | 217 | 8.89 | 5.78 | 11.35 | 88.65 |
| 0.012 mM | 50 | 4.96 | (248) | + | 1.82 | (91) | + | 0.62 | (31) | + | 7.40 | (370) | + | 363 | 14.88 | 11.76 | 48.17 | 51.83 |
| 0.025 mM | 50 | 12.66 | (633) | + | 4.36 | (218) | + | 1.56 | (78) | + | 18.58 | (929) | + | 920 | 37.70 | 34.59 | 56.31 | 43.69 |
| 0.05 mM | 50 | 20.16 | (1008) | + | 11.50 | (575) | + | 4.40 | (220) | + | 36.06 | (1803) | + | 1765 | 72.34 | 69.22 | 61.66 | 38.34 |
| *mwh/TM3* | | | | | | | | | | | | | | | | | |
| 0.006 mM | 40 | 3.03 | (121) | + | 0.65 | (26) | + | nc | | | 3.68 | (147) | + | 147 | 7.53 | 5.12 | | |
| 0.012 mM | 40 | 3.30 | (132) | + | 0.85 | (34) | + | nc | | | 4.15 | (166) | + | 166 | 8.50 | 6.10 | | |
| 0.025 mM | 40 | 7.08 | (283) | + | 1.48 | (59) | + | nc | | | 8.55 | (342) | + | 342 | 17.52 | 15.11 | | |
| 0.05 mM | 40 | 11.45 | (458) | + | 2.68 | (107) | + | nc | | | 14.13 | (565) | + | 565 | 28.94 | 26.54 | | |
| **Carboplatin** | | | | | | | | | | | | | | | | | |
| *mwh/flr*$^3$ | | | | | | | | | | | | | | | | | |
| 0.1 mM | 50 | 6.80 | (340) | + | 0.48 | (24) | + | 0.12 | (06) | i | 7.40 | (370) | + | 370 | 15.16 | 12.05 | 18.79 | 81.21 |
| 0.2 mM | 50 | 11.00 | (550) | + | 0.94 | (47) | + | 0.10 | (05) | i | 12.04 | (602) | + | 601 | 24.63 | 21.52 | 19.52 | 80.48 |
| 0.5 mM | 50 | 35.72 | (1786) | + | 2.10 | (105) | + | 0.26 | (13) | + | 38.08 | (1904) | + | 1902 | 77.95 | 74.84 | 14.84 | 85.16 |
| 1 mM | 50 | 86.98 | (4349) | + | 8.56 | (428) | + | 0.32 | (16) | + | 95.86 | (4793) | + | 4793 | 196.43 | 193.32 | 21.40 | 78.60 |
| *mwh/TM3* | | | | | | | | | | | | | | | | | |
| 0.1 mM | 40 | 5.55 | (222) | + | 0.40 | (16) | + | nc | | | 5.95 | (238) | + | 238 | 12.19 | 9.78 | | |
| 0.2 mM | 40 | 9.13 | (365) | + | 0.50 | (20) | + | nc | | | 9.63 | (385) | + | 385 | 19.72 | 17.32 | | |
| 0.5 mM | 40 | 30.45 | (1218) | + | 1.83 | (73) | + | nc | | | 32.28 | (1291) | + | 1291 | 66.14 | 63.73 | | |
| 1 mM | 40 | 69.08 | (2763) | + | 6.25 | (250) | + | nc | | | 75.33 | (3013) | + | 3013 | 154.35 | 151.95 | | |
| **Oxaliplatin** | | | | | | | | | | | | | | | | | |
| *mwh/flr*$^3$ | | | | | | | | | | | | | | | | | |
| 0.1 mM | 50 | 1.72 | (86) | – | 0.24 | (12) | i | 0.00 | (00) | – | 1.96 | (98) | – | 98 | 4.02 | 0.90 | – | – |
| 0.2 mM | 50 | 1.60 | (80) | – | 0.20 | (10) | i | 0.02 | (01) | i | 1.82 | (91) | – | 90 | 3.69 | 0.57 | – | – |
| 0.5 mM | 50 | 1.42 | (71) | – | 0.30 | (15) | i | 0.08 | (04) | i | 1.80 | (90) | – | 90 | 3.69 | 0.57 | – | – |
| 1 mM | 50 | 1.40 | (70) | – | 0.08 | (04) | – | 0.06 | (03) | i | 1.54 | (77) | – | 77 | 3.16 | 0.04 | – | – |

[a] Marker-trans-heterozygous flies (*mwh/flr*$^3$) and balancer-heterozygous flies (*mwh/TM3*) were evaluated. Negative control: distilled water. Positive control: urethane 20 mM.
[b] Statistical diagnoses according to Frei and Würgler (1988, 1995) for comparison with negative control: −, negative; i, inconclusive; +. positive ($P = 0.05$); m: minimal risk multiplication factor for the assessment of negative results.
[c] Including rare *flr*$^3$ spots.
[d] nc: not calculated. Only *mwh* single spots can be observed in *mwh/TM3* heterozygotes as the balancer chromosome *TM3* does not carry the *flr*$^3$ mutation.
[e] Considering *mwh* clones from *mwh* single and twin spots.
[f] $C = 48{,}800$ (approximate number of cells examined per fly).
[g] Induction frequencies corrected for spontaneous incidence estimated from the negative controls.
[h] Percentage of recombination (R) and mutation (M) were calculated according to Frei and Würgler (1996): R = 1-[(n/NC in *mwh/TM3* flies)/(n/NC in *mwh/flr*$^3$ flies)] * 100; M = 100-R. Control corrected frequencies were used for these calculations.

**Table 2**
SMART results in *mwh/flr*$^3$ and *mwh/TM3* progeny of the HB cross after the chronic exposure of larvae with cisplatin, carboplatin, and oxaliplatin.

| Treatments, drugs and genotypes[a] | N[o] of flies (*N*) | Spots per fly (no. of spots) statistical diagnosis[b] | | | | | | | | | | | | Spots with *mwh* | Frequency of clone formation per $10^5$ cells (*n/NC*)[f] | | Recombination[h] (%) | Mutation[h] (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Small single spots[c] (1–2 cells) *m = 2* | | | Large single spots[c] (> 2 cells) *m = 5* | | | Twin spots[d] *m = 5* | | | Total spots[c] *m = 2* | | | clone[e] (*n*) | Observed | Control Corrected[g] | | |
| Negative control *mwh/flr*$^3$ | 50 | 1.62 | (81) | | 0.16 | (08) | | 0.06 | (03) | | 1.84 | (92) | | 91 | 3.73 | | | |
| Negative control *mwh/TM3* | 40 | 0.88 | (35) | | 0.08 | (03) | | nc | | | 0.95 | (38) | | 38 | 1.95 | | | |
| Positive control *mwh/flr*$^3$ | 20 | 40.65 | (813) | + | 16.50 | (330) | + | 2.30 | (46) | + | 59.45 | (1189) | + | 1777 | 182.07 | 168.79 | | |
| **Cisplatin** | | | | | | | | | | | | | | | | | | |
| *mwh/flr*$^3$ | | | | | | | | | | | | | | | | | | |
| 0.006 mM | 50 | 5.22 | (261) | + | 1.08 | (54) | + | 0.20 | (10) | + | 6.50 | (325) | + | 324 | 13.28 | 9.55 | 31.33 | 68.67 |
| 0.012 mM | 50 | 9.28 | (464) | + | 2.16 | (108) | + | 0.54 | (27) | + | 11.98 | (599) | + | 594 | 24.34 | 20.61 | 41.35 | 58.65 |
| 0.025 mM | 50 | 21.48 | (1074) | + | 4.94 | (247) | + | 0.76 | (38) | + | 27.18 | (1359) | + | 1348 | 55.25 | 51.52 | 53.16 | 46.84 |
| 0.05 mM | 48 | 22.52 | (1081) | + | 8.52 | (409) | + | 2.35 | (113) | + | 33.40 | (1603) | + | 1594 | 68.05 | 64.32 | 53.33 | 46.67 |
| *mwh/TM3* | | | | | | | | | | | | | | | | | | |
| 0.006 mM | 40 | 3.33 | (133) | + | 0.83 | (33) | + | nc | | | 4.15 | (166) | + | 166 | 8.50 | 6.56 | | |
| 0.012 mM | 40 | 5.68 | (227) | + | 1.18 | (47) | + | nc | | | 6.85 | (274) | + | 274 | 14.04 | 12.09 | | |
| 0.025 mM | 40 | 10.63 | (425) | + | 2.10 | (84) | + | nc | | | 12.73 | (509) | + | 509 | 26.08 | 24.13 | | |
| 0.05 mM | 30 | 12.67 | (380) | + | 2.93 | (88) | + | nc | | | 15.60 | (468) | + | 468 | 31.97 | 30.02 | | |
| **Carboplatin** | | | | | | | | | | | | | | | | | | |
| *mwh/flr*$^3$ | | | | | | | | | | | | | | | | | | |
| 0.1 mM | 50 | 8.24 | (412) | + | 0.76 | (38) | + | 0.10 | (05) | i | 9.10 | (455) | + | 460 | 18.85 | 15.12 | 19.72 | 80.28 |
| 0.2 mM | 50 | 17.48 | (874) | + | 0.90 | (45) | + | 0.10 | (05) | i | 18.48 | (924) | + | 924 | 37.87 | 34.14 | 26.02 | 73.98 |
| 0.5 mM | 50 | 41.18 | (2059) | + | 3.02 | (151) | + | 0.08 | (04) | i | 44.28 | (2214) | + | 2210 | 90.57 | 86.84 | 17.06 | 82.94 |
| 1 mM | 50 | 83.44 | (4172) | + | 9.12 | (456) | + | 0.16 | (08) | i | 92.72 | (4636) | + | 4632 | 189.84 | 186.11 | 21.05 | 78.95 |
| *mwh/TM3* | | | | | | | | | | | | | | | | | | |
| 0.1 mM | 40 | 6.18 | (247) | + | 0.70 | (28) | + | nc | | | 6.88 | (275) | + | 275 | 14.09 | 12.14 | | |
| 0.2 mM | 40 | 12.05 | (482) | + | 1.23 | (49) | + | nc | | | 13.28 | (531) | + | 531 | 27.20 | 25.26 | | |
| 0.5 mM | 40 | 33.13 | (1325) | + | 2.98 | (119) | + | nc | | | 36.10 | (1444) | + | 1444 | 73.98 | 72.03 | | |
| 1 mM | 40 | 63.68 | (2547) | + | 8.98 | (359) | + | nc | | | 72.65 | (2906) | + | 2906 | 148.87 | 146.93 | | |
| **Oxaliplatin** | | | | | | | | | | | | | | | | | | |
| *mwh/flr*$^3$ | | | | | | | | | | | | | | | | | | |
| 0.1 mM | 50 | 2.72 | (136) | + | 0.42 | (21) | + | 0.06 | (03) | – | 3.20 | (160) | + | 160 | 6.56 | 2.83 | 74.64 | 25.36 |
| 0.2 mM | 50 | 1.90 | (95) | – | 0.32 | (16) | i | 0.04 | (02) | – | 2.26 | (113) | – | 113 | 4.63 | 0.90 | - | - |
| 0.5 mM | 50 | 3.16 | (158) | + | 0.30 | (15) | i | 0.06 | (03) | – | 3.52 | (176) | + | 176 | 7.21 | 3.48 | 83.82 | 16.18 |
| 1 mM | 50 | 2.30 | (115) | w+ | 0.24 | (12) | i | 0.04 | (02) | – | 2.58 | (129) | w+ | 129 | 5.29 | 1.56 | 83.55 | 16.45 |
| *mwh/TM3* | | | | | | | | | | | | | | | | | | |
| 0.1 mM | 40 | 1.10 | (44) | – | 0.20 | (08) | i | nc | | | 1.30 | (52) | – | 52 | 2.66 | 0.72 | | |
| 0.2 mM | 40 | 1.00 | (40) | – | 0.10 | (04) | i | nc | | | 1.10 | (44) | – | 44 | 2.25 | 0.31 | | |
| 0.5 mM | 40 | 1.05 | (42) | – | 0.18 | (07) | i | nc | | | 1.23 | (49) | – | 49 | 2.51 | 0.56 | | |
| 1 mM | 40 | 1.00 | (40) | – | 0.08 | (03) | – | nc | | | 1.08 | (43) | – | 43 | 2.20 | 0.26 | | |

[a] Marker-trans-heterozygous flies (*mwh/flr*$^3$) and balancer-heterozygous flies (*mwh/TM3*) were evaluated. Negative control: distilled water. Positive control: urethane 20 mM.

[b] Statistical diagnoses according to Frei and Würgler (1988, 1995) for comparison with negative control: −, negative; i, inconclusive; w+, weak positive; +. positive ($P = 0.05$); m: minimal risk multiplication factor for the assessment of negative results.

[c] Including rare *flr*$^3$ spots.

[d] nc: not calculated. Only *mwh* single spots can be observed in *mwh/TM3* heterozygotes as the balancer chromosome *TM3* does not carry the *flr*$^3$ mutation.

[e] Considering *mwh* clones from *mwh* single and twin spots.

[f] $C = 48{,}800$ (approximate number of cells examined per fly).

[g] Induction frequencies corrected for spontaneous incidence estimated from the negative controls.

[h] Percentage of recombination (R) and mutation (M) were calculated according to Frei and Würgler (1996): R = 1-[(n/NC in *mwh/TM3* flies)/(n/NC in mwh/*flr*$^3$ flies)] * 100; M = 100-R. Control corrected frequencies were used for these calculations.

chemotherapeutic drug and compared the results to the data obtained for two widely investigated agents, namely cisplatin and carboplatin. This evaluation was carried using the SMART in ST and HB crosses of *D. melanogaster*, which differ quantitatively in the activity of CYP450 enzymes. In addition, we calculated the contribution of mutation and somatic recombination to the induction of genetic damage by the three drugs comparing the results obtained for the genotypes *mwh/flr*$^3$ and *mwh/TM3*.

Similarly to results published in the literature, carboplatin and cisplatin were shown to be potent mutagens *in vivo* and *in vitro*. In this sense, it has been shown that cisplatin induced sister chromatid exchange (SCE), chromosomal aberrations, and increased micronucleus frequencies in human lymphocyte cultures as well as bone marrow cells and peripheral blood of mice and rats (Antunes et al., 2005; Khynriam and Prasad, 2003; Kosminder et al., 2004; Nersesyan et al., 2006, Oliveira et al., 2009; Rjiba-Touati et al., 2012; Serpeloni et al., 2013). Both platinum complexes were shown to promote an increase in micronucleus frequency generated by chromosome breaks and numerical chromosome aberrations in the cytokinesis block micronucleus assay (CBMN) associated with the fluorescence *in situ* hybridization (FISH) technique. Cisplatin and carboplatin also induced gene mutation in the SOS chromotest in *Escherichia coli* (Overbeck et al., 1996; Gebel et al., 1997) and the *Hprt* gene mutation assay in Chinese hamster ovary (CHO) cells (Gebel et al., 1997).

Previous studies have characterized cisplatin as a mutagenic and recombinagenic platinum complex using the wing SMART (Katz, 1987; Danesi et al., 2010a,b) and *w/w*$^+$ eye SMART (García Sar et al., 2012) in *D. melanogaster*. The compound was also shown to induce mitotic recombination in diploid cells of *Aspergillus nidulans* (Miyamoto et al., 2007). In cells of the brain ganglion of *D. melanogaster* exhibited high frequencies of double strand breaks caused by cisplatin (Garcia Sar et al., 2008, 2012).

*In vitro* studies on the effect of carboplatin in human lymphocytes induced significant increase in SCE values, chromosome aberrations, and micronucleus frequency. The compound also increased the frequency of chromosome aberrations in CHO cells (Cid et al., 1995; de Souza et al., 2017). Similar results were obtained in a study that used human lymphocytes to assess the effect of carboplatin, when the compound was shown to induce significant increase in SCE values *in vivo* and *in vitro*. Patients given a single dose of carboplatin (450 mg/m$^2$) presented increased values of these parameters, though these were still lower than the ones observed after treatment with cisplatin (Shinkai et al., 1988). In another study, carboplatin induced a dose-dependent increase in micronucleus frequency in Ehrlich ascites carcinoma (Quintana et al., 1994). In mice treated with three intraperitoneal injections of carboplatin in a 5-day treatment, micronucleus frequencies increased in a dose-dependent way in bone marrow erythrocytes (Quita et al., 2012).

Few studies have investigated the mutagenic potential of oxaliplatin. Comparing the mutagenic activity of oxaliplatin using the *Hprt* gene mutation assay in Chinese hamster ovary (CHO) cells, Silva et al., 2005 observed similar results as reported in the present study. Oxaliplatin was less mutagenic and cytotoxic than cisplatin, with a distinct mutational range. In this sense, although both platinum complexes induced preferentially the G:C→T:A transversions, oxaliplatin induced an increased rate of A:T→T:A transversions and mild deletions/insertions compared with cisplatin. De Souza et al. (2017) evaluated oxaliplatin and carboplatin through the CBMN-Cyt assay and results demonstrated that both drugs significantly increased the frequency of micronuclei (MN), nucleoplasmatic bridges (NPBs), and nuclear buds (NBUDs). They also showed that oxaliplatin induces significantly more chromosomal abnormalities than carboplatin at lower concentrations while carboplatin was more efficient than oxaliplatin in the induction of chromosomal instability events. Also, Almeida et al. (2006) used the alkaline version of the comet assay in H460 tumor cells to evaluate the formation of DNA bridges caused by oxaliplatin and cisplatin. The authors also assessed the kinetic of damage repair, and used the same version of the comet assay to assess DNA adducts in chemotherapy patients treated with oxaliplatin. The results showed that cisplatin induced higher numbers of adducts compared to *in vitro* administration of oxaliplatin using the same concentrations of the platinum complex, and exhibited different damage repair rates. Similarly, these drugs were shown to induce lesions in lymphocytes *in vivo*, though differences were observed in bridge formation and damage repair (Krüger et al., 2015). Pang et al. (2007) also used the alkaline version of the comet assay in HCT116 colorectal cancer cells and observed that only oxaliplatin and carboplatin induced DNA lesions, as opposed to cisplatin, which was not genotoxic in this system.

Chistyakov et al. (2018) showed that cisplatin and oxaliplatin were highly genotoxic to the *E. coli* recombinant lineage MG1655 pCo1D-lux using the SOS chromotest. The authors also observed differences in variety and formation rate of reactive oxygen species (ROS), and concluded that genetic damage induced by oxaliplatin in the model used were preferentially associated with the production of ROS. In turn, cisplatin induced mainly direct DNA damage measured as SOS response in *E. coli*. In this sense, Alqudah et al. (2018) and Waseem et al., 2017 evaluated the potential protective effect of vitamin E and melatonin, respectively, *in vitro* on oxaliplatin-induced DNA damage. Pretreatment with vitamin E was able to reduce chromosomal aberrations and sister chromatid exchanges induced by oxaliplatin in cultured human lymphocytes (Alqudah et al., 2018), while the administration of melatonin prior to oxaliplatin to SH-SY5Y human neuroblastoma cells also reduced the genotoxic damage of this drug, evaluated through comet assay (Waseem et al., 2017). Similar results were observed *in vivo* with male BALB/c mice that received a single dose intraperitoneal injection of oxaliplatin after ten days of treatment with the flavonoid naringenin using 8-hydroxydeoxy-guanosine marker, comet, micronucleus and chromosomal aberration assays (Ganaie et al., 2019). The protective action observed in these studies were attributed to the anti-oxidant activity of vitamin E, melatonin and naringenin, confirming that oxidative stress is involved in oxaliplatin-induced DNA damage.

Such difference in results observed in literature data, together with the results of the present study suggest that the platinum complexes assessed have distinct DNA damage induction patterns (Alcindor and Beauger, 2011; Mehmood, 2014; Chistyakov et al., 2018). These differences are associated with the types of crosslinks formed in DNA, the possibility of formation of RNA crosslinks, the induction of oxidative DNA damage, and other cell structures (Silva et al., 2005; Alcindor and Beauger, 2011). For example, concerning the interchain crosslink formation, which contributes significantly to the cytotoxicity of cisplatin, this kind of damage has been considered less important as action mechanism of oxaliplatin (Alcindor and Beauger, 2011). Therefore, the low genetic damage frequency of oxaliplatin in the SMART in *D. melanogaster*, compared with cisplatin and carboplatin, may be associated with the fact that this platinum complex induces mainly oxidative DNA damage. Additionaly, oxaliplatin did not demonstrate a genotoxic dose response relationship, differently from cisplatin and carboplatin. One of the reasons that could explain this behavior is related to the toxic effect. The results show that the concentration of 1 mM was more toxic than the concentration of 0.5 mM, which may also be reflected in a higher cytotoxicity and therefore interfere with the genotoxic effect. Additionally, the genotoxicity observed for oxalipatin is associated to metabolization, which in turn may also interfere with genotoxic activity, as several factors may interfere with metabolite generation. Guérard et al. (2015) argue that in order to evaluate the existence of non-linear dose–response relationships for genotoxicants, it is suggested that careful attention should be given to the mode of genotoxic action, relevant biomarkers of exposure, as well as to the existence and impact of potential cytoprotective mechanisms like detoxifying metabolism and DNA repair.

In this sense, hydrogen peroxide, which is used as positive control in genotoxicity studies, did either not induce oxidative DNA damage

(Sotibrán et al., 2011) or induce a low frequency of genetic damage in the SMART (Romero-Jiménez et al., 2005; Anter et al., 2010, 2011; 2014; Fernández-Bedmar et al., 2011). Also, when the effect of hydrogen peroxide in the SMART (Romero-Jiménez et al., 2005; Anter et al., 2010) is compared with the data obtained in the present study for oxaliplatin, both compounds were shown to induce preferentially damage caused by somatic recombination.

Considering the differences observed in the quantification of genetic damage based on origin (mutation or recombination) caused by cisplatin and carboplatin, cisplatin exhibited dose-dependent damage while carboplatin induced mutation lesions, independently of concentration. The literature shows that recombinational repair pathways play a key role in the repair of damage induced by cisplatin, although the nucleotide excision repair (NER) has been suggested as the main repair pathway (Zdraveski et al., 2000). In addition, platinum complexes are highly active in tumors whose cells are homologous recombination deficient (Zhao et al., 2017), which underscores the need for further studies to assess the role of homologous recombination in the repair of genetic damage caused by these drugs.

The results obtained show that oxaliplatin induces genetic damage only in the HB cross, which has higher expression of CYP450 enzymes. On the other hand, cisplatin and carboplatin did not differ in mutagenic potential when the results obtained for both crosses are compared. Previously published data show that oxaliplatin undergoes fast, far-reaching non-enzymatic biotransformation and is not metabolized by CYP450 enzymes (Graham et al., 2000). Differently from cisplatin, oxaliplatin undergoes non-enzymatic transformation in plasma, forming reactive compounds due to the release of an oxalate group from its molecule. Considering the byproducts formed, only the dichloroplatinum complexes (DACH) enter the cell, where they have cytotoxic effect (Alcindor and Beauger, 2011). Masek et al. (2009) assessed the interaction of different platinum complexes like cisplatin, carboplatin, and oxaliplatin with human CYP450 enzymes and concluded that cisplatin had weak inhibition potential against CYP2C9 and CYPP2B6, while oxaliplatin had such effect only against CYP2C9. Nevertheless, these effects were not significant in view of the low plasma concentrations of these drugs. This points to the need for more investigations for the results of the present study to be more thoroughly clarified.

As observed for genotoxic activity, the results of the toxicity of the platinum complexes studies point to differences especially between cisplatin and the other drugs (Graham et al., 2000; Mehmood, 2014; Martinez-Balibrea et al., 2015). These findings indicate that the causes of the toxic effects observed are similar and include not only DNA damage, but also the modulation of specific molecules involved in the balance between apoptosis and the maintenance of the cell cycle (Donzelli et al., 2004).

## 5. Conclusion

The results of SMART in *D. melanogaster* showed that oxaliplatin induced mutagenic effect only in the HB cross, pointing to the possible interference of CYP450 enzymes in the mutagenic action of the compound. Also, this chemotherapeutic platinum complex induced lower frequencies of genetic damage, and the damage induced was caused mainly by recombination events. On the other hand, cisplatin and carboplatin increased DNA damage in both crosses, and differed in the origin of damage. Carboplatin generated mainly mutation lesions, while cisplatin caused mostly mutation and recombination lesions.

The findings of the present study agree with previously published data that indicated the differences in genetic damage generated by the chemotherapeutic platinum complexes studied and in the DNA repair mechanisms associated. This underscores the need for further investigations on the effects of oxaliplatin as a means to assess the likely side effects of this drug in patients, especially concerning the evolution of secondary tumors.

## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## References

Alcindor, T.,, Beauger, N., 2011. Oxaliplatin: a review in the era of molecularly targeted therapy. Curr. Oncol. 18 (1), 18–25.
Ali, I., Haque, A., Wani, W.A., 2013. Platinum compounds: a hope for future cancer chemotherapy. Anti Cancer Agents Med. Chem. 13 (2), 296–306.
Almeida, G.M., Duarte, T.L., Steward, W.P., Jones, G.D.D., 2006. Detection of oxaliplatin-induced DNA crosslinks *in vitro* and in cancer patients using the alkaline comet assay. DNA Repair 5, 219–225.
Alqudah, M.A.Y., Al-Ashwal, F.Y., Alzoubi, K.H., Alkhatatbeh, M., Khabour, O., 2018. Vitamin E protects human lymphocytes from genotoxicity induced by oxaliplatin. Drug. Chem. Toxicol. 41 (3), 281–286.
Ang, W.H., Myint, M., Lippard, S.J., 2010. Transcription inhibition by platinum-DNA cross-links in live mammalian cells. J. Am. Chem. Soc. 132 (21), 7429–7435.
Anter, J., Campos-Sánchez, J., Hamss, R.E., Rojas-Molina, M., Muñoz-Serrano, A., Analla, M., Alonso-Moraga, A., 2010. Modulation of genotoxicity by extra-virgin olive oil and some of its distinctive components assessed by use of the Drosophila wing-spot test. Mutat. Res. 703 (2), 137–142.
Anter, J., Fernández-Bedmar, Z., Villatoro-Pulido, M., Demyda-Peyras, S., Moreno-Millán, M., Alonso-Moraga, A., Muñoz-Serrano, A., Luque de Castro, M.D., 2011. A pilot study on the DNA-protective, cytotoxic, and apoptosis-inducing properties of olive-leaf extracts. Mutat. Res. 723 (2), 165–170.
Anter, J., Tasset, I., Demyda-Peyrás, S., Ranchal, I., Moreno-Millán, M., Romero-Jimenez, M., Muntané, J., Luque de Castro, M.D., Muñoz-Serrano, A., Alonso-Moraga, Á., 2014. Evaluation of potential antigenotoxic, cytotoxic and proapoptotic effects of the olive oil by-product "alperujo", hydroxytyrosol, tyrosol and verbascoside. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 772, 25–33.
Antunes, L.M., Pascoal, L.M., Bianchi, M.L., Dias, F.L., 2005. Evaluation of the clastogenicity and anticlastogenicity of the carotenoid bixin in human lymphocyte cultures. Mutat. Res. 585, 113–119.
Chistyakov, V.A., Prazdnova, E.V., Mazanko, M.S., Churilov, M.N., Chmyhalo, V.K., 2018. Increase in bacterial resistance to antibiotics after cancer therapy with platinum-based drugs. Mol. Biol. 52 (2), 232–236.
Cid, M.G., Mudry, M., Larripa, I., 1995. Chromosome damage induced by carboplatin (CBDCA). Toxicol. Lett. 76, 97–103.
Danesi, C.C., Bellagamba, B.,C., Dihl, R.R., De Andrade, H.H.R., Cunha, K.S., Lehmann, M., 2010b. Evaluation of the genotoxicity of cisplatin, paclitaxel and 5-fluorouracil combined treatment in the Drosophila wing-spot test. Food Chem. Toxicol. 48, 3120–3124.
Danesi, C.C., Bellagamba, B.C., Dihl, R.R., De Andrade, H.H.R., Cunha, K.S., Spano, M.A., Reguly, M.L., Lehmann, M., 2010a. Mutagenic evaluation of combined paclitaxel and cisplatin treatment in somatic cells of *Drosophila melanogaster*. Mutat. Res. 696, 139–143.
de Souza, A.P., Lehmann, M., Dihl, R.R., 2017. Comparative study on the induction of complex genomic alterations after exposure of mammalian cells to carboplatin and oxaliplatin. Drug. Chem. Toxicol. 40, 410–415.
Deepa, P., Kolandaivel, P., Senthilkumar, K., 2013. Structural properties and the effect of platinum drugs with DNA base pairs. Struct. Chem. 24, 583–595.
Donzelli, E., Carfì, M., Miloso, M., Strada, A., Galbiati, S., Bayssas, M., Griffon-Etienne, G., Cavaletti, G., Petruccioli, M.G., Tredici, G., 2004. Neurotoxicity of platinum compounds: comparison of the effects of cisplatin and oxaliplatin on the human neuroblastoma cell line SH-SY5Y. J. Neuro Oncol. 67 (1–2), 65–73.
Fernández-Bedmar, Z., Anter, J., de La Cruz-Ares, S., Muñoz-Serrano, A., Alonso-Moraga, A., Pérez-Guisado, J., 2011. Role of citrus juices and distinctive components in the modulation of degenerative processes: genotoxicity, antigenotoxicity, cytotoxicity, and longevity in Drosophila. J. Toxicol. Environ. Health A. 74 (15–16), 1052–1066.
Francesco, A.M.D.I., Ruggiero, A., Riccardi, R., 2002. Cellular and molecular aspects of drugs of the future: oxaliplatin. Cell. Mol. Life Sci. 59, 1914–1927.
Frei, H., Clements, J.J., Hove, D., Würgler, F.E., 1992. The genotoxicty of the nticancer drug mitoxantrone in somatic and germ-cells of *Drosophila melanogaster*. Mutat. Res. 279, 21–33.
Frei, H., Würgler, F.E., 1988. Statistical methods to decide whether mutagenicity test data from Drosophila assays indicate a positive, negative, or inconclusive result. Mutat.

Res. 203 (4), 297–308.
Frölich, A., Würgler, F.E., 1989. New tester strains with improved bioactivation capacity for the Drosophila wing-spot test. Mutat. Res. 216, 179–187.
Frei, H., Würgler, F.E., 1995. Optimal experimental design and sample size for the statistical evaluation of data from somatic mutation and recombination tests (SMART) in Drosophila. Mutat. Res. 334 (2), 247–258.
Frei, H., Würgler, F.E., 1996. Induction of somatic mutation and recombination by four inhibitors of eukaryotic topoisomerases assayed in the wing spot test of *Drosophila melanogaster*. Mutagenesis 11, 315–325.
Ganaie, M.A., Jan, B.L., Khan, T.H., Alharthy, K.M., Sheikh, I.A., 2019. The protective effect of naringenin on oxaliplatin-induced genotoxicity in mice. Chem. Pharm. Bull. 67 (5), 433–438.
García Sar, D., Montes-Bayón, M., Aguado Ortiz, L., Blanco-González, E., Sierra, L.M., Sanz-Medel, A., 2008. *In vivo* detection of DNA adducts induced by cisplatin using capillary HPLC-ICP-MS and their correlation with genotoxic damage in *Drosophila melanogaster*. Anal. Bioanal. Chem. 390, 37–44.
García Sar, D., Aguado, L., Montes Bayón, M., Comendador, M.A., Blanco González, E., Sanz-Medel, A., Sierra, L.M., 2012. Relationships between cisplatin-induced adducts and DNA strand-breaks, mutation and recombination *in vivo* in somatic cells of *Drosophila melanogaster*, under different conditions of nucleotide excision repair. Mutat. Res. 741 (1–2), 81–88.
Gebel, T., Lantzsch, H., Plebow, K., Dunkelberg, H., 1997. Genotoxicity of platinum and palladium compounds in human and bacterial cells. Mutat. Res. 389, 183–190.
Graf, U., Frei, H., Kägi, A., Katz, A.J., Würgler, F.E., 1989. Thirty compounds tested in the Drosophila wing spot test. Mutat. Res. 222, 359–373.
Graf, U., van Schaik, N., 1992. Improved high bioactivation cross for the wing somatic mutation and recombination test in *Drosophila melanogaster*. Mutat. Res. 271, 59–67.
Graf, U., Würgler, F.E., Katz, A.J., Frei, J., Juon, H., Hall, C.B., Kale, P.G., 1984. Somatic mutation and recombination test in *Drosophila melanogaster*. Environ. Mol. Mutagen. 6, 153–188.
Graham, M.A., Lockwood, G.F., Greenslade, D., Brienza, S., Bayssas, M., Gamelin, E., 2000. Clinical pharmacokinetics of oxaliplatin: a critical review. Clin. Cancer Res. 6 (4), 1205–1218.
Guérard, M., Baum, M., Bitsch, A., Eisenbrand, G., Elhajouji, A., Epe, B., Habermeyer, M., Kaina, B., Martus, H.J., Pfuhler, S., Schmitz, C., Sutter, A., Thomas, A.D., Ziemann, C., Froetschl, R., 2015. Assessment of mechanisms driving non-linear dose-response relationships in genotoxicity testing. Mutat. Res. Mutat Res. 763, 181–201.
Hostetter, A.A., Chapman, E.G., Derose, V.J., 2009. Rapid cross-linking of an rna internal loop by the anticancer drug cisplatin. J. Am. Chem. Soc. 131, 9250–9257.
Jung, Y., Lippard, S.J., 2007. Direct cellular responses to platinum-induced DNA damage. Chem. Rev. 107, 1387–1407.
Kastenbaum, M.A., Bowman, K.O., 1970. Tables for determining the statistical significance of mutation frequencies. Mutat. Res. 9 (5), 527–549.
Katz, A.J., 1987. Genotoxic effects of cisplatin in somatic tissue of *Drosophila melanogaster*. Environ. Mol. Mutagen. 10, 197–203.
Khynriam, D., Prasad, S.B., 2003. Cisplatin-induced genotoxic effects and endougenous glutathione levels in mice bearing ascites dalton's lymphoma. Mutat. Res. 526, 9–18.
Kosminder, B., Wyszynska, K., Janik-Spiechowicz, R., Osiecka, R., Ochocki, E., Ciesielska, E., Wasowicz, W., 2004. Evaluation of the genotoxicity of cis-bis-(3-aminoflavone) dichloroplatinum(II) in comparison with cis-DDP. Mutat. Res. 558, 93–110.
Krüger, K., Thomale, J., Stojanović, N., Osmak, M., Henninger, C., Bormann, S., Fritz, G., 2015. Platinum-induced kidney damage: unraveling the DNA damage response (DDR) of renal tubular epithelial and glomerular endothelial cells following platinum injury. Biochim. Biophys. Acta. 1853 (3), 685–698.
Lindsley, D.L., Zimm, G.G., 1992. The Genome of *Drosophila melanogaster*. Academic Press Inc., San Diego.
Martinez-Balibrea, E., Martínez-Cardús, A., Ginés, A., Ruiz de Porras, V., Moutinho, C., Layos, L., Manzano, J.L., Bugés, C., Bystrup, S., Esteller, M., Abad, A., 2015. Tumor-related molecular mechanisms of oxaliplatin resistance. Mol. Cancer Ther. 14 (8), 1767–1776.
Masek, V., Anzenbacherová, E., Machová, M., Brabec, V., Anzenbacher, P., 2009. Interaction of antitumor platinum complexes with human liver microsomal cytochromes P450. Anti Canccer Drugs 20 (5), 305–311.
Mehmood, R.K., 2014. Review of Cisplatin and oxaliplatin in current immunogenic and monoclonal antibody treatments. Onco Rev. 8 (2), 256.
Misiak, M., Mantegazza, F., Beretta, G.L., 2016. Methods for elucidation of DNA-anticancer drug interactions and their applications in the development of new drugs. Curr. Pharmaceut. Des. 22 (44), 6596–6611.
Miyamoto, C.T., Sant'anna, J.R., Franco, C.C.S., Castro-Prado, M.A.A., 2007. Genotoxicity (mitotic recombination) of the cancer chemotherapeutic agents cisplatin and cytosine arabinoside in aspergillus nidulans. Food Chem. Toxicol. 45, 1091–1095.
Ndinguri, M.W., Solipuram, R., Gambrell, R.P., Aggarwal, S., Hansel, W., Hammer, R.P., 2009. Peptide targeting of platinum anti-cancer drugs. Bioconjug. Chem. 20, 1869–1878.
Nersesyan, A., Perrone, E., Roggier, P., Bolognesi, C., 2006. Cytogenetic toxicity of cycloplatam in human lymphocytes: detection by the micronucleus test and fluorescence in situ hybridization. Anti Canccer Drugs 17, 289–295.
Oliveira, R.J., Baise, E., Mauro, M.O., Pesarini, J.R., Matuo, R., Ribeiro, L.R., Mantovani, M.S., 2009. Evaluation of chemopreventive activity of glutamine by the comet and the micronucleus assay in mice's peripheral blood. Environ. Toxicol. Pharmacol. 28, 120–124.
Overbeck, T.L., Knight, J.M., Beck, D.J., 1996. A comparison of the genotoxic effects of carboplatin and cisplatin in Escherichia coli. Mutat. Res. 362, 249–259.
Pang, S.K., Yu, C.W., Au-Yeung, S.C., Ho, Y.P., 2007. DNA damage induced by novel demethylcantharidin-integrated platinum anticancer complexes. Biochem. Biophys. Res. Commun. 363, 235–240.
Quintana, E., Pertusa, J., Gonzales, R., Renau-Piqueras, J., 1994. Carboplatin treatment induces dose-dependent increases in the frequency of micronuclei in Erlich ascites tumor cells. Mutat. Res. 322, 55–60.
Quita, S., Mutawakal, M.Z., Kurdi, L., Hifni, B., 2012. Mutagenic effects of carboplatin on somatic cells of male albino mice. Arab Gulf J. Sci. Res. 30, 164–171.
Rjiba-Touati, K., Ayed-Boussema, I., Skhiri, H., Belarbia, A., Zellema, D., Achour, A., Bacha, H., 2012. Induction of DNA fragmentation, chromosome aberrations and micronuclei by cisplatin in rat bone-marrow cells: protective effect of recombinant human erythropoietin. Mutat. Res. 747, 202–206.
Romero-Jiménez, M., Campos-Sánchez, J., Analla, M., Muñoz-Serrano, A., Alonso-Moraga, A., 2005. Genotoxicity and anti-genotoxicity of some traditional medicinal herbs. Mutat. Res. 585 (1–2), 147–155.
Serpeloni, J.M., Almeida, M.R., Mercadante, A.Z., Bianchi, M.L.P., Antunes, L.M.G., 2013. Effects of lutein and chlorophyll b on GSH depletion and DNA damage induced by cisplatin *in vivo*. Hum. Exp. Toxicol. 32, 828–836.
Shinkai, T., Saijo, N., Eguchi, K., 1988. Cytogenetic effect of carboplatin on human lymphocytes. Cancer Chemother. Pharmacol. 21, 203–207.
Silva, M.J., Costa, P., Dias, A., Valente, M., Louro, H., Boavida, M.G., 2005. Comparative analysis of the mutagenic activity of oxaliplatin and cisplatin in the *Hprt* gene of CHO cells. Environ. Mol. Mutagen. 46 (2), 104–115.
Sotibrán, A.N., Ordaz-Téllez, M.G., Rodríguez-Arnaiz, R., 2011. Flavonoids and oxidative stress in *Drosophila melanogaster*. Mutat. Res. 726 (1), 60–65.
Souza, C., Villarino, N.F., Farnsworth, K., Black, M., 2017. Enhanced cytotoxicity of bleomycin, cisplatin, and carboplatin on equine sarcoid cells following electroporation-mediated delivery *in vitro*. J. Vet. Pharmacol. Ther. 40 (1), 97–100.
Spreckelmeyer, S., Orvig, C., Casini, A., 2014. Cellular transport mechanisms of cytotoxic metallodrugs: an overview beyond cisplatin. Molecules 19 (10), 15584–15610.
Waseem, M., Sahu, U., Salman, M., Choudhury, A., Kar, S., Tabassum, H., Parvez, S., 2017. Melatonin pre-treatment mitigates SHSY-5Y cells against oxaliplatin induced mitochondrial stress and apoptotic cell death. PLoS One 12 (7), e0180953.
Wheate, N.J., Walker, S., Craig, G.F., Oun, R., 2010. The status of platinum anticancer drugs in the clinic and in clinical trials. Dalton Trans. 39 (3), 8113–8127.
Wu, B., Davey, G.E., Nazarov, A.A., Dyson, P.J., Davey, C.A., 2011. Specific DNA structural attributes modulate platinum anticancer drug site selection and cross-link generation. Nucleic Acids Res. 39, 8200–8212.
Zdraveski, Z.Z., Mello, J.A., Marinus, M.G., Essigmann, J.M., 2000. Multiple pathways of recombination define cellular responses to cisplatin. Chem. Biol. 7 (1), 39–50.
Zhao, E.Y., Shen, Y., Pleasance, E., Kasaian, K., Leelakumari, S., Jones, M., Bose, P., Ch'ng, C., Reisle, C., Eirew, P., Corbett, R., Mungall, K.L., Thiessen, N., Ma, Y., Schein, J.E., Mungall, A.J., Zhao, Y., Moore, R.A., Brok, W.D., Wilson, S., Villa, D., Shenkier, T., Lohrisch, C., Chia, S., Yip, S., Gelmon, K., Lim, H., Renouf, D., Sun, S., Schrader, K.A., Young, S., Bosdet, I., Karsan, A., Laskin, J., Marra, M.A., Jones, S.J.M., 2017. Homologous recombination deficiency and platinum-based therapy outcomes in advanced breast cancer. Clin. Cancer Res. 23 (24), 7521–7530.