**UNIVERSIDADE FEDERAL DE UBERLÂNDIA**
**INSTITUTO DE BIOTECNOLOGIA**
**PÓS-GRADUAÇÃO EM BIOTECNOLOGIA**

**NAYARA JÚNIA DE SOUZA BONTEMPO**

# AVALIAÇÃO DO COMPLEXO METÁLICO TERNÁRIO DE COBRE ASSOCIADO A 2-(4-FLUOROFENOXI), ACETO-HIDRAZIDA, PERCLORATO E 1,10 FENANTROLINA (DRI-12) EM CÉLULAS SOMÁTICAS DE *DROSOPHILA MELANOGASTER*

**PATOS DE MINAS – MG**
**FEVEREIRO DE 2020**

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Dissertação de mestrado apresentada ao Programa de Pós-graduação em Biotecnologia como requisito parcial para obtenção do título de Mestre em Biotecnologia.

**Orientador: Prof. Dr. Robson José de Oliveira Júnior**

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**PATOS DE MINAS – MG**

**FEVEREIRO DE 2020**

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B722
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Bontempo, Nayara Junia de Souza, 1992-
Avaliação do complexo metálico ternário de cobre associado a 2-(4-fluorofenoxi), aceto-hidrazida, perclorato e 1,10 fenantrolina (DRI-12) em células somáticas de Drosophila melanogaster. [recurso eletrônico] / Nayara Junia de Souza Bontempo. - 2020.

Orientador: Robson José de Oliveira Júnior.
Coorientadora: Thaise Gonçalves de Araújo.
Dissertação (Mestrado) - Universidade Federal de Uberlândia, Pós-graduação em Biotecnologia.
Modo de acesso: Internet.
Disponível em: http://doi.org/10.14393/ufu.di.2020.269
Inclui bibliografia.
Inclui ilustrações.

1. Biotecnologia. I. Oliveira Júnior, Robson José de ,1984-, (Orient.). II. Araújo, Thaise Gonçalves de,1984-, (Coorient.). III. Universidade Federal de Uberlândia. Pós-graduação em Biotecnologia. IV. Título.

CDU: 60

Bibliotecários responsáveis pela estrutura de acordo com o AACR2:
Gizele Cristine Nunes do Couto - CRB6/2091
Nelson Marcos Ferreira - CRB6/3074

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| | | | | | |
|---|---|---|---|---|---|
| Programa de Pós-Graduação em: | Biotecnologia | | | | |
| Defesa de: | Dissertação de Mestrado Acadêmico | | | | |
| Data: | 14 de fevereiro de 2020 | | 14:00h | Hora de encerramento: | 17:00h |
| Matrícula do Discente: | 41812BTC008 | | | | |
| Nome do Discente: | Nayara Júnia de Souza Bontempo | | | | |
| Título do Trabalho: | **AVALIAÇÃO DO COMPLEXO METÁLICO TERNÁRIO DE COBRE ASSOCIADO A 2-(4-FLUOROFENOXI), ACETO-HIDRAZIDA, PERCLORATO E 1,10 FENANTROLINA (DRI-12) EM CÉLULAS SOMÁTICAS DE *Drosophila melanogaster*** | | | | |
| Área de concentração: | Biociências | | | | |
| Linha de pesquisa: | Prospecção de produtos bioativos de interesse médico e industrial | | | | |
| Projeto de Pesquisa de vinculação: | Avaliação da atividade citotóxica, antitumoral e genotóxica de 4 complexos metálicos de Cobre(ii) | | | | |

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Documento assinado eletronicamente por **Nayane Moreira Machado**, **Usuário Externo**, em 14/02/2020, às 17:30, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

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“Se eu pudesse deixar algum presente a você, deixaria aceso o sentimento de amar a vida dos seres humanos. A consciência de aprender tudo o que foi ensinado pelo tempo a fora. Lembraria os erros que foram cometidos para que não mais se repetissem. A capacidade de escolher novos rumos. Deixaria para você, se pudesse, o respeito aquilo que é indispensável. Além do pão, o trabalho. Além do trabalho, a ação. E, quando tudo mais faltasse, um segredo: o de buscar no interior de si mesmo a resposta e a força para encontrar a saída.’’

Mahatma Gandhi

Dedico essa conquista **aos meus pais**, que fizeram dos seus sonhos a realização dos meus. Obrigada por acreditarem em mim, a vocês meu infinito amor e eterna gratidão!

## AGRADECIMENTOS

Primeiramente, agradeço a **Deus** por ter me dado a vida, toda força, coragem, fé e sabedoria para que eu pudesse concluir mais esse ciclo e por ter colocado tantas pessoas especiais no meu caminho.

Ao meu **orientador**, **Robson José de Oliveira Júnior**, agradeço pela confiança em me orientar, pelo apoio, parceria e conhecimento compartilhado na consolidação desse trabalho.

A minha coorientadora, **Thaise Gonçalves de Araújo**, parabéns pela competência e maestria em orientar, seus ensinamentos foram vastos. Agradeço por confiar em mim, dedicar o seu tempo na correção dessa dissertação, contribuindo com sugestões para o engrandecimento da mesma. Você me fez crescer como pessoa e profissional.

A **Priscila Capelari Orsolin**, obrigada por ter me acolhido desde a graduação e ter me apoiado em todos os momentos até aqui. Sua contribuição foi além do mundo acadêmico. A você serei eternamente grata!

A **Universidade Federal de Uberlândia** e os **docentes do Programa de Pós Graduação em Biotecnologia (Patos de Minas)**, obrigada pela participação significativa na minha formação.

Ao **Centro Universitário de Patos de Minas (UNIPAM)** por tornar viável a realização dos experimentos.

Aos membros da banca examinadora, **Alexandre Azenha Alves de Rezende** e **Nayane Moreira Machado**, por se disponibilizarem a contribuir com este trabalho.

Ao Prof. Dr. **Ulrich Graf** do Instituto de Toxicologia da Universidade de Zurich Schwerzenbach, Suíça, pelo fornecimento da linhagem mutante *multiple wing hairs* de *Drosophila melanogaster*.

Ao **Bloomington *Drosophila* Stock Center**, da Universidade de Indiana nos Estados Unidos (USA), pelo fornecimento da linhagem mutante *wts* de *Drosophila melanogaster*.

Aos **colegas de Mestrado**, pela amizade e momentos compartilhados.

A todos do **Laboratório de Citogenética e Mutagênese (LABCIM – UNIPAM)**, pela cumplicidade, apoio e incentivo quando surgiam obstáculos e problemas nos experimentos. A viabilidade desse trabalho só se tornou possível graças ao auxílio e empenho de todos.

Ao **Gbio** pela receptividade e boa convivência.

Ao secretário do Programa de Pós Graduação em Biotecnologia, **Aparecido**, obrigada pela disponibilidade em ajudar.

Aos meus pais, **João e Ediva**, por todo o suporte em minha vida, o verdadeiro amor incondicional. Obrigada pelos valores transmitidos, pelo carinho, apoio e confiança na minha capacidade. Vocês serão minha eterna inspiração.

Ao meu irmão, **Diego,** pelo incentivo, exemplo de determinação. Agradeço por estar presente em todos os momentos da minha vida.

Ao meu namorado, **Jean**, meu presente de Deus, obrigada pela compreensão, paciência e por ter sido meu porto seguro mesmo nos momentos mais difíceis. O seu amor e companheirismo me fizeram mais forte nessa caminhada.

A **Paula Marynella**, uma irmã de alma que o mestrado me deu. Obrigada pelas experiências trocadas e pela amizade verdadeira. Você é uma pessoa admirável!

A todos os **meus amigos**, em especial ao Matheus Soares, Brendhal Almeida, Matheus Fernandes, Samuel, Amanda Dias, Douglas Brandão, Adriana Dias, Natália Cristina, Valquíria Rodrigues, Mariana Ribeiro, Jéssica Machado, Ketlely, e Franciele

Marinho, vocês trouxeram mais luz, força e determinação para minha vida. Agradeço por não me deixarem desistir.

A todos os **meus familiares**, por torcerem pelo meu sucesso, sobretudo minha **prima** Simone e minha **tia** Geni pela presença constante.

A todos que de forma direta ou indireta contribuíram para a realização deste sonho, muito obrigada! Vocês me mostraram que quando se têm objetivos é preciso ter foco. As dificuldades passam, as lutas sempre existirão, mas a vitória é certa para aqueles que não desistem e colocam **DEUS** à frente de tudo.

## SUMÁRIO


INTRODUÇÃO ...... 13

Capítulo formatado de acordo com as normas da Associação Brasileira de Normas Técnicas – ABNT. ...... 15

CAPÍTULO I ...... 15

REVISÃO DA LITERATURA ...... 15

2.1 Câncer: definição e dados epidemiológicos ...... 16

2.2 O tratamento quimioterápico e os complexos metálicos ...... 19

2.3 *Drosophila melanogaster* como modelo experimental ...... 23

2.3.1 Teste para detecção de mutação e recombinação somática (SMART) em asas de *Drosophila melanogaster* ...... 25

2.3.2 Teste para detecção de tumores epiteliais (ETT) em *Drosophila melanogaster* ...... 28

REFERÊNCIAS ...... 31

CAPÍTULO II ...... 39

Abstract ...... 42

1. INTRODUCTION ...... 43

2. MATERIAL and METHODS ...... 45

2.1 DRI-12 and Doxorubicin ...... 45

2.2 Toxicity test in *Drosophila melanogaster* ...... 46

2.4 Epithelial Tumor Test (ETT) in *Drosophila melanogaster* ...... 50

3. RESULTS ...... 51

4. DISCUSSION ...... 61

5. CONCLUSION ...... 66

REFERENCES ...... 67

**Lista de Figuras**

**Figura 1.** Taxas de incidência do câncer estimadas para 2018/2019 por sexo, no mundo. ....................................................................................................18

**Figura 2.** Representação da estrutura química do complexo metálico. ....................23

**Figura 3.** Casal de *Drosophila melanogaster*: O macho (esquerda) é menor e apresenta o pente sexual. A fêmea (direita) é maior. ...............................................24

**Figura 4.** Ciclo de vida da mosca *Drosophila melanogaster*. ...................................25

**Figura 5.** Pelos *mwh* e *flr*$^{3}$ de *Drosophila melanoga*ster. Fotomicrografia, microscópio óptico de luz (aumento de 400x) apresentando em (A) pelos *multiple wing hairs* e em (B) pelos *flare*. .........................................................................27

**Figura 6.** Esquema representativo do cruzamento ST realizado com as linhagens do teste SMART. Aspectos fenotípicos dos descendentes MH e BH gerados e eventos genéticos identificados. ..........................................................................................28

**Figura 7.** Tumor no corpo da *Drosophila melanogaster.* ..........................................29

**Figura 8.** Esquema representativo do cruzamento entre as linhagens *multiple wing hairs* e a linhagem *wts* e seus descendentes do teste ETT. ....................................30

## Lista de Tabelas

**Table 1.** Results obtained in the marked trans-heterozygous (MH) descendants of *Drosophila melanogaster* derived from the standard (ST) cross and high bioactivation (HB) crosss treated with different DRI-12 concentratios, negative control (reverse osmosis water) and positive control (0.4mM Doxorrubicin). ......................................54

**Table 2.** Results obtained in the marked trans-heterozygous descendants (MH) and balancer-heterozygous (BH) of *Drosophila melanogaster* derived from the DRI-12 associated with Doxorubicin (0.4mM) and negative control (reverse osmosis water). ..........................................................................................................................56

**Table 3.** Tumor clone frequency observed in the co-treatment in *Drosophila melanogaster*, heterozygote for the *wts* tumor suppressor gene, treated with doxorrubicin and different concentrations of DRI -12. ..............................................58

**Table 4.** Tumor clone frequency observed in the post-treatment in *Drosophila melanogaster*, heterozygote for the *wts* tumor suppressor gene, post-treated with doxorrubicin and different concentrations of DRI -12. ..............................................60

## Lista de abreviaturas e símbolos

| | |
|---|---|
| **BH** | Heterozigotos balanceados |
| **Cu (II)** | Cobre oxidado |
| **Cu (I)** | Cobre reduzido |
| ***Cyp6A2*** | Enzimas P450 |
| **DRI -12** | Complexo ternário (2-(4-fluorofenoxi) aceto-hidrazida)-bis (perclorato)-(1,10-fenantrolina)-cobre (II) |
| **DNA** | Ácido Desoxirribonucléico |
| **DDT** | *(*Dicloro-difenil-tricloroetano) |
| **ERRO** | Espécies reativas de oxigênio |
| ***flr³*** | Linhagem *flare* 3 de *Drosophila melanogaster* |
| **HB** | Cruzamento de alta bioativação |
| **$H_2O_2$** | Peróxido de hidrogênio |
| **GSH** | Glutationa peroxidase |
| **GTS** | Glutationa S-transferase |
| **MH** | Trans-heterozigotos marcados |
| ***mwh/mwh*** | Linhagem *multiple wing hairs* de *Drosophila melanogaster* |
| **$O_2^-$** | Superóxido |
| **$OH^-$** | Radical Hidroxila |
| ***ORR*** | Linhagem Oregon R, *flr³* de *Drosophila melanogaster* |
| **Phen** | Fenantrolina |
| **SMART** | Teste de mutação e recombinação somática |
| **SOD** | Superóxido dismutase |
| **ST** | Cruzamento padrão |

## Resumo

O câncer é a segunda maior causa de morte no mundo sendo que, anualmente, cerca de 18 milhões de pessoas são diagnosticadas com algum tipo da doença. Tratamentos direcionados e novos fármacos têm aumentado a sobrevida dos pacientes. Contudo, quadros de resistência e comorbidades ainda são frequentes, evidenciando a necessidade da busca por compostos mais seletivos, menos tóxicos e capazes de combater a resistência das células tumorais. Nesse sentido, este estudo objetivou avaliar o potencial mutagênico, recombinogênico, carcinogênico e/ou anticarcinogênico de um composto de cobre associado a 2-(4-fluorofenoxi), aceto-hidrazida, perclorato e 1,10 fenantrolina (DRI-12). Para isso, foram realizados experimentos *in vivo* em *Drosophila melanogaster:* o teste para detecção de clones de tumores epiteliais (ETT), para a avaliação do potencial anticarcinogênico, e o Teste de Mutação e Recombinação Somática (SMART), para verificar a mutagenicidade/recombinogenicidade do complexo metálico. As concentrações (0,015; 0,031; 0,062; 0,125 e 0,250mM) foram estabelecidas com base no teste de toxicidade (TX) sendo que essas foram utilizadas isoladamente e em associação com Doxorrubicina (DXR). Com relação ao teste SMART, o DRI-12 isolado não apresentou potencial mutagênico/recombinogênico. Já no tratamento associado à DXR houve redução na frequência de manchas em todas as concentrações testadas, quando comparada ao controle positivo (DXR a 0,4mM). No teste ETT (co e pós-tratamento) foi evidente a ausência de efeito carcinogênico e a atividade moduladora do composto DRI-12 sobre a ação da DXR, uma vez que também houve redução estatisticamente significativa ($p<0.05$) na frequência de tumores, quando comparada ao controle positivo. Nossos resultados, portanto, sugerem um novo composto metálico como promissor no tratamento do câncer, sendo necessário validá-lo nos diferentes tipos tumorais.

**Palavras-chave**: Câncer. Cobre. DRI-12. *Drosophila melanogaster.*

## Abstract

Cancer is the second leading cause of death in the world, and about 18 million people are diagnosed with some type of this disease. Targeted treatments and new drugs increase patient’s survival. However, resistance and comorbidities are still frequent, highlighting the need for more selective compounds, less toxic and capable of combating the resistance of tumor cells. In this sense, this study aimed to evaluate the mutagenic, recombinogenic, carcinogenic and/or anticarcinogenic potential of a copper compound associated with 2- (4-fluorophenoxy), acetohydrazide, perchlorate and 1.10 phenanthroline (DRI-12). In vivo experiments were carried out on *Drosophila melanogaster*, which are: the test for detection of epithelial tumor clones (ETT), to assess the anticarcinogenic potential, and the somatic mutation and recombination test (SMART), to verify the mutagenicity/recombinogenicity of the metal complex. The samples, alone and in association with Doxorubicin (DXR), were used at 0.015; 0.031; 0.062; 0.125 and 0.250mM, based on the toxicity test (TX). Regarding the SMART test, DRI-12 did not show mutagenic/recombinogenic potential. In the treatment associated with DXR, there was a reduction in the frequency of spots in all concentrarions tested, when compared to the positive control (DXR at 0.4mM). For ETT test (co and post-treatment) there was no carcinogenic effect and the modulating activity of the compound DRI-12 on the action of DXR was evident, since the tumors significantly reduced ($p<0.05$) when compared to positive control. Our results suggest a new promising metallic compound in the treatment of cancer, being necessary to validate for different tumor types.

**Keywords**: Cancer. Copper. DRI-12. *Drosophila melanogaster*.

# INTRODUÇÃO

O câncer é uma doença multicausal crônica, caracterizada pelo crescimento descontrolado de células transformadas. Estas alterações são decorrentes de danos em genes envolvidos no controle do ciclo celular e apoptose (Plankar e Jerman, 2011), incluindo os proto-oncogenes e os genes supressores de tumores (Dornelas *et al.*, 2016). Tal patologia é considerada um dos maiores problemas da saúde pública.

Aproximadamente 14 milhões de pessoas são diagnosticadas todo ano (OMS, 2017). As opções para o tratamento incluem, em sua maioria, a remoção cirúrgica do tumor (em casos de tumores sólidos), radioterapia, imunoterapia, hormonioterapia, ablação térmica e quimioterapia. Este último combate sistemicamente às células malignas mediante administração de um ou mais medicamentos (Sudhakar, 2009). Contudo, os quimioterápicos não apresentam a seletividade necessária, causando a concomitante lesão de células normais (Barry e Sadler, 2013; Medici *et al.*, 2015; Cheff e Hall, 2017). São observados, portanto, efeitos adversos durante o tratamento como dor, fadiga, falta de apetite, náuseas e vômitos, inchaço, problemas intestinais, depressão, susceptibilidade a infecções e urticária (INCA, 2012; De Souza Rodrigues e Polidori, 2012).

Nesse contexto, a descoberta de novas drogas se mostra, de fato, necessária. Estas podem ser utilizadas junto aos compostos tradicionais, reduzindo os efeitos adversos, ou como nova estratégia de tratamento sistêmico, justificando o presente estudo. Sendo assim, esse trabalho tem como objetivo avaliar o potencial antitumoral do complexo ternário (2-(4-fluorofenoxi) aceto-hidrazida)-bis (perclorato)-(1,10-fenantrolina)-cobre (II), também chamado de DRI-12, formado a partir da associação de aceto-hidrazida, perclorato e 1,10 fenantrolina ao cobre (II). Hipotetiza-se que, considerando o efeito farmacológico e anticancerígeno desempenhado pelos metais essenciais, como o cobre, o DRI-12 seja uma substância promissora no combate a células tumorais. Sugere-se que o composto metálico não apresente potencial carcinogênico, mutagênico/recombinogênico em *Drosophila melanogaster* para que, assim, possa ser utilizado e validado em diferentes modelos tumorais.

# CAPÍTULO I
## REVISÃO DA LITERATURA

*Capítulo formatado de acordo com as normas da Associação Brasileira de Normas Técnicas – ABNT.*

*2.1 Câncer: definição e dados epidemiológicos*

A informação genética está inscrita no DNA, devidamente organizada de modo a garantir sua transmissibilidade. Alterações em sua sequência são conhecidas como mutações genéticas que, apesar de necessárias para o processo evolutivo, também podem ocasionar proliferação desorganizada, acúmulo de erros e o aparecimento de tumores (ACS, 2017).

Nas mutações gênicas, quando pontuais, podem acontecer substituições, inserções ou deleções de apenas uma base nitrogenada ou um par de nucleotídeos. Funcionalmente, podem ser subdivididas em missense, nonsense, silenciosa e *frameshift*. Mutações missense ocorrem quando a troca nucleotídica também modifica o aminoácido codificado, alterando a cadeia polipeptídica. Mutações nonssense, ou sem sentido, decorrem da ocorrência prematura de um códon de terminação, resultando em uma proteína que, por vezes, não é funcional. Quando a mudança no material genético não altera a sequência de aminoácidos do polipeptídeo, a mutação é dita silenciosa. Isso só é possível porque o código genético é degenerado e mais de um códon codifica para o mesmo aminoácido. Por fim, mutações *frameshift* ocorrem quando a adição ou remoção de uma base altera as trincas ou quadro de leitura, culminando com uma tradução drasticamente alterada (Gorlov *et al.*, 2018; Hildebrand *et al.*, 2019).

As células apresentam mecanismos de reparo para detectar e corrigir os danos ao DNA. Dessa forma, durante o ciclo celular existem pontos de verificação (*checkpoints*) antes da divisão e distribuição do material genômico das células mitóticas para as células filhas (Reinhardt e Yaffe, 2013). Quando ocorre a detecção de algum erro, há uma pausa na replicação, para que ocorra o reparo do DNA ou a indução de morte programada (Reinhardt e Schumacher, 2012). No entanto, a perpetuação de danos conduz à transformação maligna e à resistência terapêutica. Portanto, repará-los é essencial na prevenção e no prognóstico da doença (Roos *et al.*, 2016).

De modo geral, as vias de reparo de DNA podem atuar por meio da reversão direta do dano no DNA ou da excisão completa dessa lesão. Essas se dividem em: reparo de mal pareamento (MMR, do inglês: *Mismatch Repair*), reparo por excisão de bases (BER, do inglês: *Base Excision Repair*) e nucleotídeos (NER, do inglês: *Nucleotide Excision Repair*), reparo por recombinação homóloga (HR, do inglês:

*Homologous Recombination*), junção de extremidades não-homólogas (NHEJ, do inglês: *Non-Homologous End Joining*) e via translesão ou mutagênica de reparo (TLS, do inglês: *Translesion Synthesis*). Apesar de apresentadas individualmente, evidências apontam para *crosstalk* e a sobreposição desses mecanismos no processamento de lesões ao DNA (Fu *et al.*, 2012; Haynes *et al.*, 2015; Malaquin *et al.*, 2015; Simonelli *et al.*, 2017).

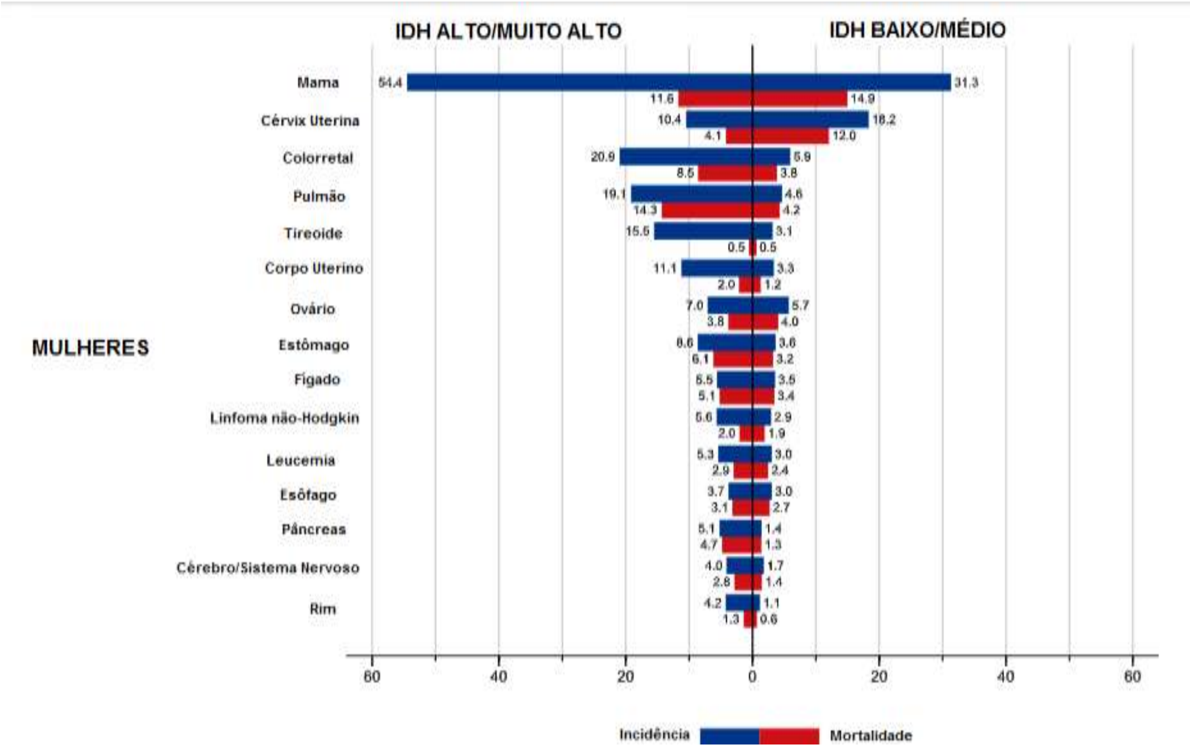
A mutação no DNA é um passo crítico na tumorigênese. Quando acontece em genes supressores de tumor ou proto-oncogenes as consequências são drásticas. Responsáveis por controlar o ciclo celular e sinalizar para a apoptose, esses genes coordenam a homeostase do organismo em um contínuo balanço entre morte e proliferação (Kang *et al.*, 2017).

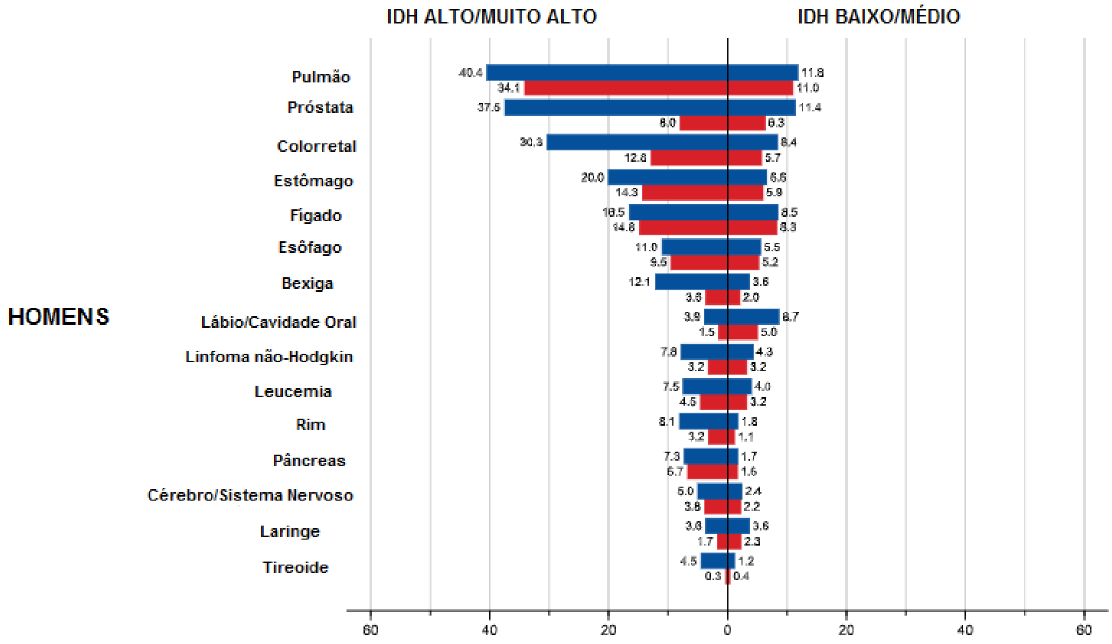
Mecanismos intrínsecos (como erros durante o processo replicativo) e condições adversas conduzem a danos na molécula do DNA. Nesse cenário, é importante ressaltar o papel dos agentes químicos, físicos, biológicos; além da dieta inadequada e sedentarismo como responsáveis por alterações gênicas e consequente tumorigênese. Esses podem conduzir a danos oxidativos que, se não reparados, contribuem para a promoção de um ambiente transformado (Wiseman e Halliwell, 1996; Resende, 2007). De fato, encontram-se descritas marcas ou eventos moleculares associados ao surgimento e progressão do câncer. Além da desregulação metabólica, destacam-se a sustentação dos sinais proliferativos, inibição dos supressores tumorais, evasão à morte celular, imortabilidade replicativa, evasão ao sistema imune, instabilidade genômica, indução da angiogênese e metástase (Chatterjee e Walker, 2017).

O câncer engloba mais de 100 tipos de doenças (Dantas *et al.*, 2009) e, apesar dos avanços na compreensão de suas bases moleculares, as taxas de recidiva permanecem elevadas, o que o torna um problema de saúde pública. Esta realidade está diretamente associada à presença de micrometástases e células tumorais circulantes, as quais são continuamente liberadas para a colonização de sítios distantes (Birkenkamp-Demtröder, 2018).

É considerado também a segunda principal causa de morte no mundo, sendo responsável por 9,6 milhões de mortes em 2018. Globalmente, um em cada seis óbitos relacionam-se a neoplasias. Aproximadamente 70% dessas mortes ocorrem em países de baixa e média rendas (OPAS, 2018, OMS 2019). Em termos de Brasil, de acordo com dados do INCA, são estimados 625 mil novos casos de câncer para o triênio 2020-2022 (INCA, 2020). A organização Mundial de Saúde (OMS) estimou que, no ano de 2030, espera-se 27 milhões de casos incidentes, 17 milhões de mortes e 75 milhões de diagnósticos (INCA, 2018). A figura1 mostra as taxas dos tipos de Cânceres no mundo decorrentes em 2018.

**Figura 1.** Taxas de incidência do câncer estimadas para 2018/2019 por sexo, no mundo.






**Fonte:** Adaptado de Bray et al. (2018).

A alta mortalidade associa-se, frequentemente, ao diagnóstico tardio. Além disso, muitos pacientes sucumbem durante o tratamento, tornando esses índices ainda mais alarmantes. Nesse contexto, prevenir a doença permanece como a melhor estratégia. A prevenção primária envolve a adoção de hábitos saudáveis e a secundária a vigilância epidemiológica com a realização de exames periódicos (Marsicano *et al.*, 2015). Importantes programas nacionais de rastreio estão em vigor buscando conscientizar a população quanto à importância da periodicidade desses exames (Babayan e Pantel, 2018; Lee *et al.*, 2019). Além disso, as pesquisas por novas estratégias de tratamento também se mostram particularmente interessantes, visando sobretudo, uma melhor qualidade de vida dos pacientes.

*2.2 O tratamento quimioterápico e os complexos metálicos*

Em geral, em oncologia, são utilizados quatro tipos de tratamento: cirurgia, radioterapia, imunoterapia e quimioterapia. A quimioterapia inclui a administração sistêmica, em geral intravenosa, de medicamentos capazes de combater micrometástases clinicamente indetectáveis após a cirurgia (WHO, 2006). De acordo com as suas finalidades, o tratamento quimioterápico é classificado em: curativo, adjuvante, neoadjuvante e paliativo. A quimioterapia curativa é indicada como estratégia de controle completo do tumor. A adjuvante é realizada após a retirada cirúrgica ou após radioterapia e na ausência de metástases detectáveis. A neoadjuvante, prévia ou citorredutora, é utilizada antes da excisão cirúrgica ou radioterapia, com a finalidade de reduzir o risco de metástases ou promover a redução de tumores. Já a paliativa visa o controle de sintomas e melhora da qualidade de vida do paciente (Ferrari *et al.*, 2012).

Contudo, apesar das alterações metabólicas e bioquímicas das células tumorais serem alvos dos quimioterápicos, estes compostos apresentam fatores limitantes, pois carecem de seletividade, atingindo também células normais, o que culmina com os efeitos colaterais debilitantes aos pacientes (Park *et al.*, 2009). Além disso, os tumores podem apresentar resistência ao processo terapêutico. A primeira causa relacionada à falha terapêutica resulta de fatores genéticos. Já o tratamento medicamentoso também pode conduzir à resistência secundária ou adquirida, comprometendo a resposta do paciente. Ambos decorrem de mutações no genoma, com modificação no padrão protéico, no funcionamento de enzimas de síntese de nucleotídeos, na composição de microtúbulos, na topoisomerase ou nas vias de sinalização intracelular, ao direcionar receptores de tirosina quinase (JG Marin *et al.*, 2012; Rebucci e Michiels, 2013).

Os complexos metálicos já são utilizados como fármacos no tratamento de várias doenças, como artrite reumatoide, câncer, hipertensão, leishmaniose e infecções da pele decorrentes de queimaduras (Berman, 2006; Rollas e Küçükgüzel, 2007; Iakovidis *et al.*, 2011). O cobre, ouro, complexos de prata e os complexos de platina se destacam. Além disso, complexos de paládio e rutênio também têm se mostrado promissores no tratamento de neoplasias (Benite, 2007; Wang *et al.*, 2016).

Alguns fármacos possuem ligantes inorgânicos com grupos funcionais característicos (Bloemink e Reedijk, 1996; Benite, 2007). Um exemplo bem conhecido é o *cis*- [(diaminodicloro) platina (II)], *cis*-[Pt (NH3)2Cl2], ou simplesmente cisplatina. Este é um dos compostos mais utilizados no tratamento do câncer, cuja atividade farmacológica foi descoberta em 1965 por Rosenberg e colaboradores. Desde então, as pesquisas e desenvolvimento de fármacos empregando íons metálicos se intensificaram e novos modelos foram surgindo ao longo dos anos, melhorando a qualidade de vida de pessoas acometidas por diversas enfermidades (Rosenberg *et al.*, 1969; Sekhon e Gandhi, 2006; Silva *et al.*, 2011).

Atualmente, a cisplatina é utilizada em aproximadamente 60% dos regimes quimioterápicos, sendo eficiente no combate de diferentes tipos de neoplasias, incluindo cérebro, rim, cérvix, ovário, pulmão, estômago, esôfago, mama, cabeça, pescoço, testículo, linfomas, osteossarcoma, melanoma, leucemia (Benite, 2007; Rios e Antunes, 2009; Dasari e Tchounwou, 2014; Cheff e Hall, 2017). Além da cisplatina, dois outros complexos à base de platina nomeados diamino (1,1-ciclobutanodicarboxilato) platina (II) (carboplatina) e trans-1,2-diaminocicloexanooxaloplatina (II) (oxaliplatina) também são empregados e apresentam-se menos nefrotóxicos, o que tem permitido o uso de doses maiores. Entretanto, são menos eficientes que a cisplatina (Neves e Vargas, 2011; Warad *et al.*, 2013; Medici *et al.*, 2015). Conhecer o mecanismo de ação farmacológica se mostra, portanto, crucial para o desenvolvimento de tratamentos mais eficazes e seguros ao paciente. Quando a platina se liga ao DNA, por exemplo, causa a torção da molécula, inibindo a transcrição e provocando a morte das células tumorais (Gowda *et al.*, 2014).

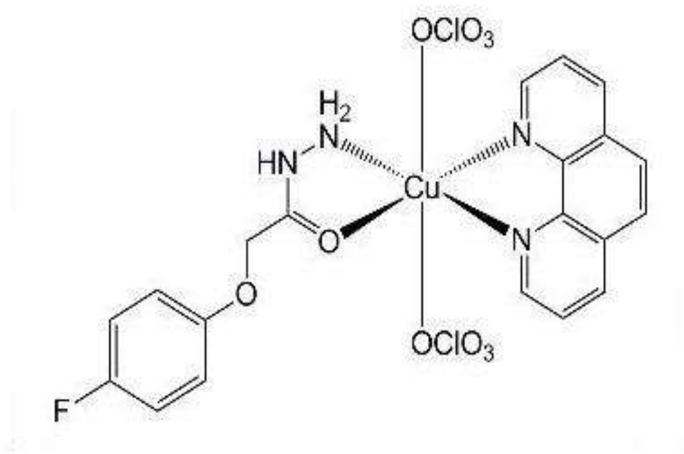
Os íons metálicos são importantes por estarem envolvidos com funções específicas que mantêm o equilíbrio dos organismos vivos, incluindo o transporte de oxigênio pelas hemácias, regulação do metabolismo da glicose e participação em complexos enzimáticos (Maret, 2016). As propriedades cinéticas e termodinâmicas dos complexos metálicos lhes conferem características peculiares diretamente relacionadas às suas atividades antimicrobianas, antivirais, anti-inflamatórias, antitumorais e de inibição enzimática (Iakovidis *et al.*, 2011; Vincent *et al.*, 2016; Othmani *et al.*, 2018).

A absorção dos íons ocorre no estômago e na primeira porção do intestino delgado, complexado à albumina, proteínas transcupreína de alto peso molecular e histidina. Íons cobre podem assumir os estados oxidado cúprico [Cu (II)] e cuproso [Cu (I)], agindo como um cofator catalítico na atividade redox de enzimas como a citocromo oxidase e a superóxido dismutase (SOD). Também participa da absorção de ferro, da respiração mitocondrial, da remoção de radicais livres e da produção de espécies reativas de oxigênio (ROS) ao reagir com o oxigênio molecular e peróxido de hidrogênio. Nesse contexto, o cobre em quantidades moderadas apresenta atividade antioxidante ao sequestrar elétrons livres e mudar de estado. Contudo, em altas concentrações pode se mostrar pró-oxidante ao gerar radicais livres (Lushchak, 2014; Twomey *et al.*, 2017).

ROS são pequenas moléculas com vida curta e altamente reativas. Podem ser radicais livres derivados de oxigênio, como o ânion superóxido ($O_2^-$) e o radical hidroxila ($OH^-$) ou moléculas não radicais, como peróxido de hidrogênio ($H_2O_2$). A geração de ROS nas células existe em equilíbrio com os mecanismos de defesas antioxidantes. Estes incluem eliminadores enzimáticos, como SOD, catalase, glutationa peroxidase e peroxirredoxinas, bem como não enzimáticos, como vitaminas C e E, glutationa (GSH), ácido lipóico, carotenóides e ferro quelantes. Em doses baixas, os ROS são considerados essenciais para a regulação das funções fisiológicas normais envolvidas no desenvolvimento como progressão e proliferação, diferenciação, migração e morte celulares. Os ROS também desempenham um papel importante na resposta imune e na manutenção do sistema redox. Além disso, já foram relacionados à ativação de vias de sinalização celular, fatores de transcrição e apoptose (Redza-Dutordoir e Averill-Bates, 2016). O excesso de ROS promove danos no DNA, culminando com o surgimento e proliferação de células neoplásicas (Sun *et al.*, 2004; Fruehauf e Meyskens, 2007; Hecht *et al.*, 2016). Nestas os mecanismos de reparo encontram-se, portanto, comprometidos, suprimindo o sinal apoptótico.

Nesse sentido, o complexo de cobre associado a 2-(4-fluorofenoxi), aceto-hidrazida, perclorato e 1,10 fenantrolina (DRI-12) (Figura 2) sintetizado na Universidade Federal de Uberlândia tem sido investigado como agente quimioterápico (Paixão *et al.*, 2017). As hidrazidas (R-CO-NH-NH2) apresentam amplo espectro de atividades biológicas, tais como antitumoral, antioxidante, antibacteriana e anti-inflamatória e a fenantrolina pode inibir o crescimento celular, o que torna o protótipo particularmente promissor (Narang *et al.*, 2012; Bingul *et al.*, 2016).

**Figura 2.** Representação da estrutura química do complexo metálico.




**Fonte**: Paixão *et al*., (2017).

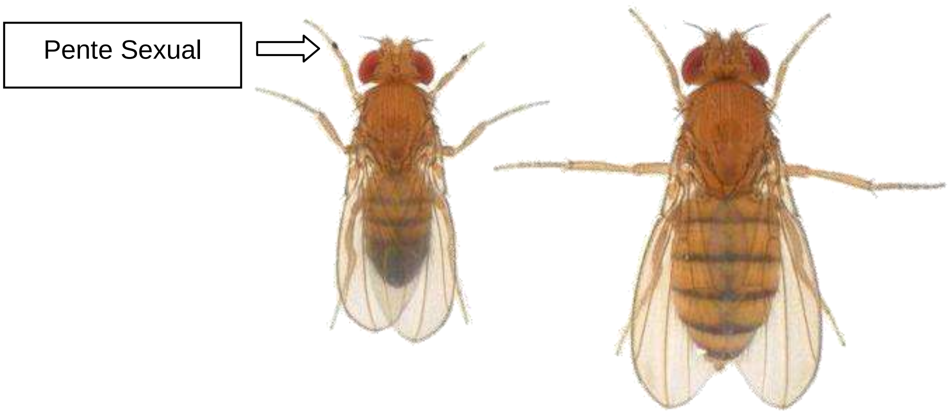
A fenantrolina apresenta atividade antiproliferativa, decorrente de sua estrutura que favorece a interação com o DNA e ao anel aromático fundido ao complexo (Ruiz-Azuara e Bravo-Gomez, 2010; Zhou *et al.*, 2011). Existem evidências em linhagens celulares (HeLa) de que a associação dos complexos de cobre com 1,10 fenantrolina estimula sua ação e citotoxidade (Kljun e Turel, 2017). Esta classe de compostos tem a capacidade de coordenar prontamente muitos metais de transição. Assim sendo, vários complexos de hidrazida já foram sintetizados e caracterizados (Rodrigues *et al.*, 2015; Sousa *et al.*, 2015).

Os complexos de cobre (II) contendo hidrazidas e ligantes N, N-heterocíclico e os seus possíveis efeitos já foram avaliados contra leucemia mielóide crônica, sendo capazes de entrar nas células e inibir seu crescimento de forma dependente da concentração (Dasari, Tchounwou, 2014). Tal atividade foi maior em associação do que quando os ligantes se encontravam livres (Silva *et al*., 2014), pois complexados conferiram maior seletividade ao composto ao modular as propriedades redox do cobre (Ruiz- Azuara e  Bravo-Gomez, 2010).

*2.3 Drosophila melanogaster como modelo experimental*

A espécie *Drosophila melanogaster* (Figura 3), conhecida popularmente como mosca da fruta, é utilizada em pesquisas genéticas desde 1909. Sua fácil manipulação em laboratório, ciclo de vida curto (cerca de 10 dias a 25º C), estrutura do genoma relativamente simples, baixo custo, manutenção simples, reações enzimáticas semelhantes as dos mamíferos e elevada progênie, a tornam atraente como modelo *in vivo*. Na forma adulta, possui cerca de 2 mm de comprimento, três pares de pernas e apenas um par de asas, uma vez que o segundo par de asas foi modificado e se encontra no interior de pequenos apêndices chamados halteres. Estes auxiliam na aerodinâmica para o voo (Snustad e Simmons, 2006).

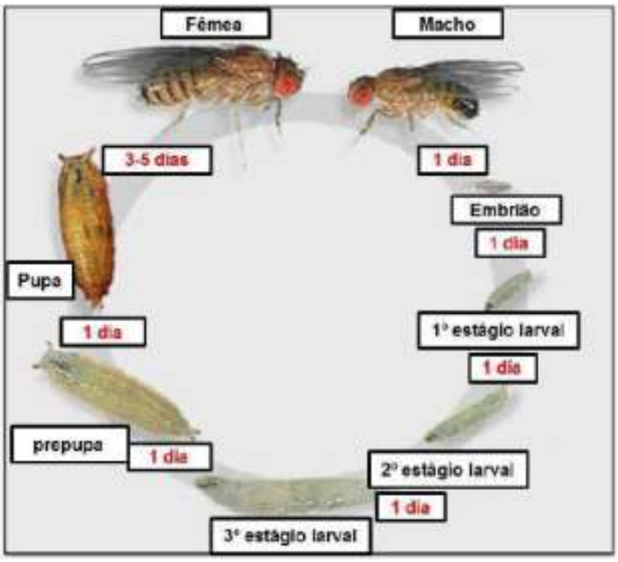
**Figura 3.** Casal de *Drosophila melanogaster*: O macho (esquerda) é menor e apresenta o pente sexual. A fêmea (direita) é maior.




**Fonte**: http://seresmodelicos.csic.es/galeria/mosca.html.

Durante seu desenvolvimento (Figura 4), o zigoto se transforma em larva de primeiro instar, que é móvel e se alimenta rapidamente de frutas maduras e fermentos, por exemplo. À medida que a larva cresce, ultrapassa sua cutícula de quitina, abandonando-a e desenvolvendo uma nova. Esse é o segundo instar. Um maior crescimento e desenvolvimento resultam em outra cutícula para produzir a larva de terceiro instar. Esse estágio aparece por volta de cinco dias após a fertilização. O corpo da larva diminui e a cutícula se espessa e acumula pigmento, caracterizando a pupa. Já as estruturas adultas, como cabeça, asas e pernas, desenvolvem-se no envoltório pupal, presentes na larva como pequenos agrupamentos chamados discos imaginais (Eeken *et al*., 2002).

**Figura 4.** Ciclo de vida da mosca *Drosophila melanogaster*.




**Fonte**: http://www.sc.didaxis.pt/hereditariedade/drosophila.html

Cerca de 80% dos genes dessa mosca são homólogos aos de humano e, adicionalmente, um número significante destes incluem genes supressores tumorais (Graf e Van Schaik, 1992; Griffiths *et al*., 2006). Portanto, verifica-se a relevância dos testes de mutagenicidade, recombinação e carcinogenicidade utilizando *D. melanogaster*, uma vez que este modelo possibilita identificar o possível efeito, *in vivo*, que alguns compostos podem provocar às células saudáveis e tumorais, em testes de co e pós-tratamento.

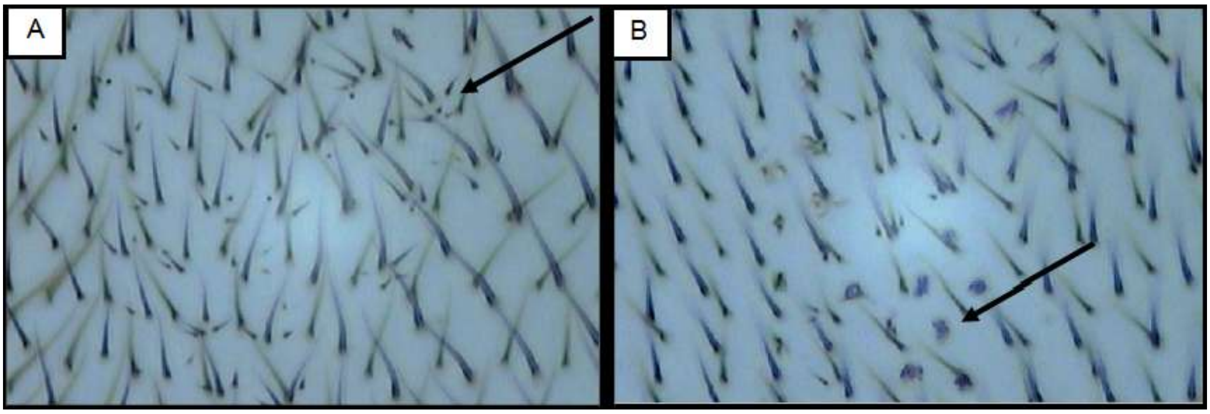
*2.3.1 Teste para detecção de mutação e recombinação somática (SMART) em asas de Drosophila melanogaster*

O teste SMART (*Somatic Mutation and Recombination Test*) é utilizado na detecção simultânea de mutações e recombinações somáticas por meio da expressão fenotípica em asas de moscas, após a perda de heterozigose em genes específicos (Machado, 2013). O teste possui uma alta eficiência para detectar a atividade genotóxica de mutágenos pertencentes a diferentes classes químicas incluindo agentes alquilantes e promutágenos, ativados por diferentes vias metabólicas de biotransformação enzimática. A versatilidade do procedimento experimental permite testar tanto compostos estáveis quanto instáveis (Graf *et al.*, 1984; Rand, 2010).

No teste para detecção de mutação e recombinação somática em células de *D. melanogaster* são utilizadas três linhagens:

- *Linhagem multiple wing hairs* (*mwh/mwh*): os indivíduos dessa linhagem apresentam o gene marcador *mwh* no cromossomo 3 (3-0,3) em uma posição distal. Quando esse alelo mutante está presente, as células da asa de *D. melonogaster*, que normalmente caracterizam-se por apresentar um único pelo, apresentarão dois ou mais pelos por célula (Figura 5, A). Por ser uma mutação viável, a linhagem estoque é mantida em homozigose recessiva (GRAF *et al*., 1984).
- *Linhagem flare 3 (*$flr^3$*)*: os indivíduos dessa linhagem possuem o gene marcador $flr^3$ localizado no cromossomo 3 (3-38,8) em uma posição mais proximal em relação ao centrômero. O fenótipo provocado por esse alelo mutante é um pelo mal formado que se assemelha a uma chama (Figura 5, B). O gene marcador $flr^3$ é letal em homozigose, não se desenvolvendo até a fase adulta. Devido à letalidade, esse alelo é mantido na linhagem estoque na presença de um balanceador cromossômico (*TM3*, $Bd^S$), que se caracteriza por múltiplas inversões (GRAF *et al*., 1984).
- *Linhagem ORR* (*Oregon R, flare*$^3$): os indivíduos que se enquadram nessa linhagem possuem a mesma constituição genética dos indivíduos que pertencem à linhagem *flare*$^3$, porém, diferem por apresentarem os cromossomos 1 e 2 provenientes da linhagem Oregon R resistente ao DDT (Dicloro-difenil-tricloroetano), com alta expressividade das enzimas citocromo P450 (Graf e Van Schaik, 1992). Essa linhagem de *D. melanogaster* foi criada com o objetivo de aumentar o desempenho do SMART de asas no caso da ativação de pró-mutágenos dependentes de ativação via citocromo P450 (GRAF *et al*., 1989).

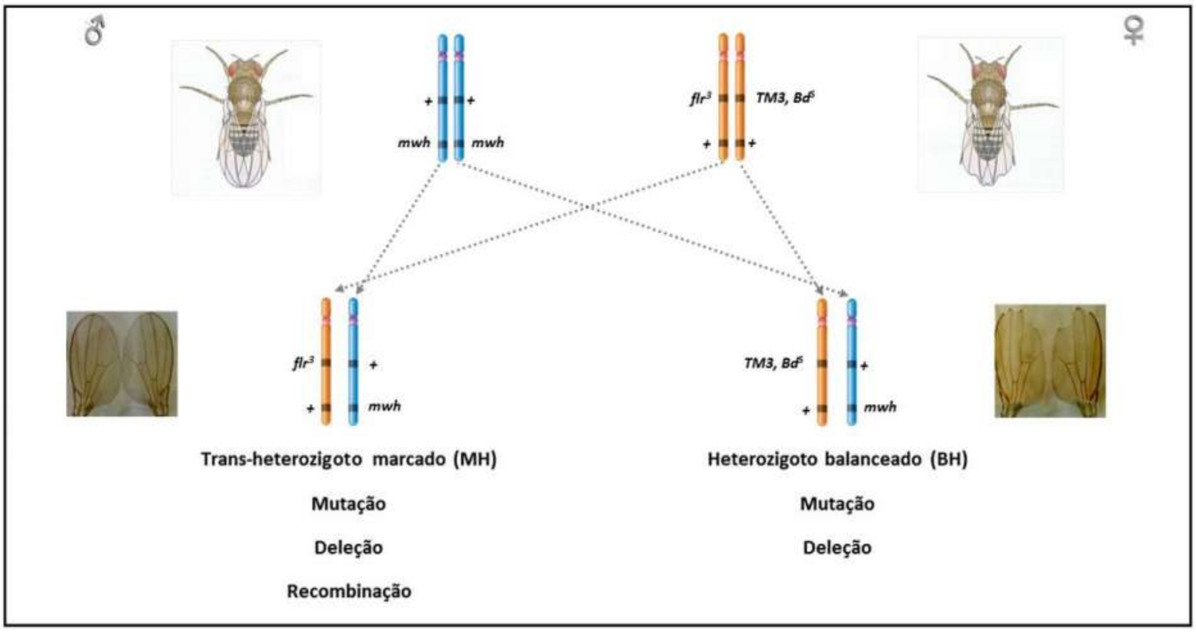
**Figura 5**. Pelos *mwh* e *flr*$^3$ de *Drosophila melanogaster*. Fotomicrografia, microscópio óptico de luz (aumento de 400x) apresentando em (A) pelos *multiple wing hairs* e em (B) pelos *flare*.




**Fonte**: Orsolin (2011).

O cruzamento padrão do SMART, também chamado cruzamento ST (*standart*) apresenta um nível basal de enzimas P450 (*Cyp6A2*), o que possibilita analisar a ação direta do composto. Este ocorre entre machos *mwh*/*mwh* e fêmeas *flr*$^3$ na presença de um balanceador cromossômico (*TM3*, *Bd*$^S$). O cruzamento de alta bioativação HB (*high bioactivation) possui* altos níveis de P450 o que favorece analisar a ação direta do composto depois de metabolizado (Figura 6). Os machos *mwh*/*mwh* são acasalados com fêmeas ORR *(Oregon R, flr*$^3$*).* O propósito da metabolização é detoxicar. Contudo, os metabólitos transformados podem se mostrar prejudiciais (Frölich e Würgler, 1989; Graf e Van Schaik, 1992; Saner *et al.*, 1996; Orsolin *et al.*, 2016).

**Figura 6.** Esquema representativo do cruzamento ST realizado com as linhagens do teste SMART. Aspectos fenotípicos dos descendentes MH e BH gerados e eventos genéticos identificados.




**Fonte**: Moraes (2015).

A atividade recombinogênica de genotoxicinas é determinada pelas análises dos descendentes, trans-heterozigotos marcados (*mwh* +/+ *flr*$^3$) (MH) e heterozigotos balanceados (*mwh* +/ *TM3, Bd*$^S$) (BH), os quais apresentam o fenótipo borda lisa e borda serrilhada da asa, respectivamente. A progênie MH pode apresentar o fenótipo *mwh* ou *flare* (manchas simples), fenótipo *mwh* e *flare* (manchas gêmeas), ocasionados por eventos mutagênicos ou recombinogênicos. Já na progênie BH todos os eventos recombinogênicos são inviabilizados devido ao cromossomo balanceador, sendo assim, apenas o fenótipo *mwh* pode ser identificado (manchas simples) (Graf et al., 1984; Graf et al., 1992).

*2.3.2* Teste para detecção de tumores epiteliais (ETT) em *Drosophila melanogaster*

O ETT é bastante utilizado para avaliar a atividade carcinogênica ou anticarcinogênica de diferentes compostos/substâncias. Trata-se de uma metodologia sensível a diferentes tratamentos e, sobretudo, econômica. Existem vários protocolos publicados com o teste, incluindo terapias simples e combinadas, em estratégias com cotratamento e pós-tratamento (Nepomuceno, 2015).

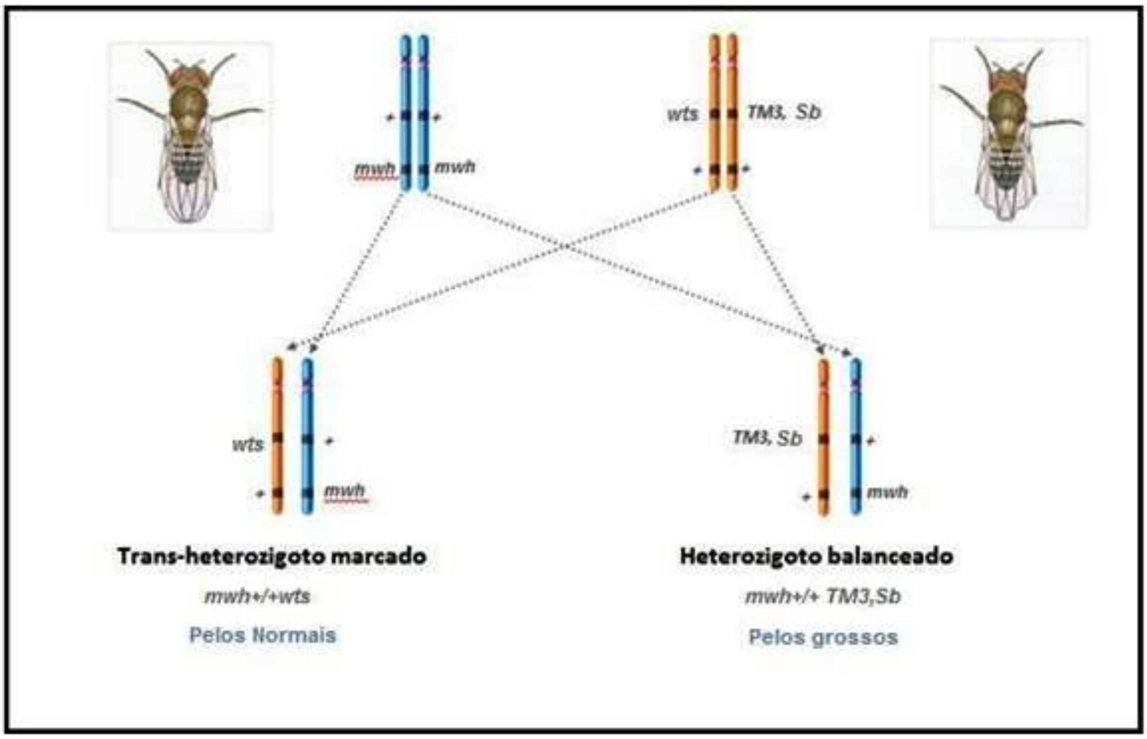
A conservação evolutiva de genes supressores tumorais entre *D. melanogaster* e mamíferos tem direcionado os estudos com essas moscas. Diversos proto-oncogenes e supressores tumorais de mamíferos também são identificados nessas espécies (Eeken *et al.*, 2002). Nishiyama *et al*. (1999) descreveram, como homólogos, o gene supressor de tumor warts (*wts*) em *D. melanogaster* com o *LATS1* (*Large tumor suppressor kinase* 1) em humanos. Quando deficiente, conduz ao desenvolvimento de sarcomas em tecidos moles e tumores ovarianos. O gene *LATS1* codifica uma proteína que apresenta um domínio serina / treonina quinase localizado no aparelho mitótico. Esse grupo de enzimas é capaz de fosforilar proteínas envolvidas no controle do ciclo celular, tais como quinases e ciclinas (NCBI, 2018). A deleção do gene *wts* acarreta, nas moscas, a formação de clones de células que são consideravelmente invasivas e com capacidade de se desenvolver por todo o corpo do indivíduo. Assim, o gene warts é considerado importante no controle da morfogênese e proliferação celular em *D. melanogaster* (Hib e Robertis, 2006). Além disso, a inativação de ambos os alelos *wts*, em todas as células da mosca, resultam em letalidade embrionária. Entretanto, a mutação e recombinação mitótica, nos indivíduos heterozigotos, podem levar a clones mutantes que induzem a formação de tumores (Figura 7) (Eeken *et al.*, 2002).

**Figura 7.** Tumor no corpo da *Drosophila melanogaster.*

**Fonte:** Arquivo pessoal (2019).

Como em homozigose, o marcador *wts* é uma mutação recessiva letal nos zigotos, esse é mantido na linhagem estoque com a presença de um balanceador cromossômico (*TM3*). Machos e fêmeas são colocados juntos (para acasalamento) para obtenção de larvas heterozigotas (*wts* +/+ *mwh*), sendo realizado o cruzamento entre fêmeas virgens *wts/TM3*, Sb1 com machos *mwh/mwh*. Por meio do cruzamento (Figura 8) entre essas linhagens são obtidas as larvas heterozigotas (*mwh/wts*) ou (*mwh/TM3*), utilizadas nos experimentos. Nos indivíduos adultos, obtidos dos tratamentos, a presença ou ausência do supressor tumoral (*wts*) é realizada pela diferenciação do tipo de pelo presente no corpo e cabeça da mosca, onde os indivíduos *mwh/wts* apresentam pelos longos e finos e os indivíduos *mwh/TM3* apresentam pelos curtos e grossos. Apenas moscas com o genótipo *mwh/wts* são analisadas. Indivíduos de genótipo *mwh/TM3* são descartadas, por não apresentarem o gene em estudo (*wts*) (Sidorov *et al.*, 2001).

**Figura 8.** Esquema representativo do cruzamento entre as linhagens *multiple wing hairs* e a linhagem *wts* e seus descendentes do teste ETT.




**Fonte**: Adaptado de Rezende (2012); Moraes (2015).

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# CAPÍTULO II

*Capítulo formatado de acordo com as normas da Food and Chemical Toxicology.*

# EVALUATION OF A NEW TERNARY COPPER METALLIC COMPLEX ASSOCIATED WITH 2-(4- FLUOROPHENOXY), ACETO HYDRAZIDE, PERCHLORATE AND 1,10 PHENANTHROLINE (DRI-12) ON SOMATIC CELLS OF *DROSOPHILA MELANOGASTER*

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**Abstract**

Copper complexes have shown antitumoral efficacy and low toxity, being a promising class of metallodrugs for the treatment of neoplasms. The present study aimed to evaluate the mutagenic/recombinogenic and anticarcinogenic potential of a copper compound associated with 2-(4-fluorophenoxy), acetohydrazide, perchlorate and 1,10 phenanthroline (DRI-12) in *Drosophila melanogaster*. For this, the Somatic Mutation and Recombination Test (SMART) and the Epithelial Tumor Test (ETT) were performed. Sub-toxic concentrations of DRI-12, defined by the toxicity test, were used alone and in association with Doxorubicin (DXR). In the SMART test, DRI-12 did not show mutagenic/recombinogenic potential, when compared to the negative control (reverse osmosis water). When associated with DXR, there was a reduction in the frequency of mutant spots, when compared to the positive control (DXR - 0.4mM). There was no carcinogenic effect in the ETT (co and post-treatment). Furthermore, the results suggested modulating activity of the prototype against DXR. Therefore, DRI-12 is relevant as a possible metalodrug for the treatment of tumors.



**Keywords:** Cancer. Chemotherapy. Copper. DRI-12. *Drosophila melanogaster*. Doxorubicin.

# 1. INTRODUCTION

The understanding of pharmacological properties of different substances, associated with the improvement of laboratory techniques related to bioinorganic, allowed the industrial scale synthesis of molecules with anti-inflammatory, anticancer, and antibacterial properties. Thus, it became possible to search for the effective treatment of chronic diseases, such as cancer (Santini et al., 2014).

Cancer results from genetic mutations which give cells the unlimited proliferation capacity, loss of response to growth inhibiting factors, apoptosis avoidance, immune escape, metabolic modulation, invasive potential, and induction of angiogenesis (Martinez et al., 2006). The systemic chemotherapy treatment, although able to control the disease, causes debilitating side effects, besides having its effectiveness reduced in the face of resistance mechanisms, with compromised clinical status of patients (Barry and Sadler, 2013; Cheff and Hall, 2017; Medici et al., 2015). Cancer is the second leading cause of death in the world, totaling 9.6 million deaths in 2018. Globally, one in six deaths is related to this disease (OMS, 2019; OPAS, 2018).Therefore, it is evident the need for new drugs for the treatment of tumors (Vega et al., 2017).

Metal complexes have been highlighted due to their promising biological effects, in addition to the physical-chemical properties of their transition metals. Metal ions, such as Iron ($Fe^{3+}$) and Copper ($Cu^{2+}$), are essential to living organisms and, in this context, subject to modifications in the development of less toxic and more selective prototypes (Baran, 2004). Copper is a metal found in nature and essential to enzymes that act in the antioxidant defense, a fact that makes it feasible for use in metal complexes. Additionally, there are evidences that copper is capable to induce DNA cleavage and nucleic base oxidation by producing reactive oxygen species (ROS) (Tardito and Marchio, 2009; Twomey et al., 2017). The cytotoxicity of this metal and its ability to inhibit the growth of tumor cells has already been described (Paixão et al., 2017), however, not yet widely explored.

For the synthesis of metal complexes, there are three main points: the presence of an essential metal which reduces toxicity; the cis configuration around the metal ion and the different scale of hydrophobicity present in the complex, favoring the absorption and distribution of the drug by the cells (Ruiz-Azuara and E Bravo-Gomez, 2010). Paixão and contributors synthesized in 2017 the copper complex associated with 2-(4-fluorophenoxy), acetohydrazide, perchlorate and 1,10 phenanthroline (DRI-12) (Paixão et al., 2017). Its hydrazides (R-CO-NH-$NH_2$) have a wide spectrum of biological activities, such as antitumoral, antioxidant, antibacterial and anti-inflammatory, and the phenanthroline is able to inhibit cell growth (Bingul et al., 2016; Narang et al., 2012).

However, it is known that some prototypes may have mutagenic and/or carcinogenic potential, whereas others can mitigate these effects. For this reason, many compounds have been and continue to be tested in different experimental systems (Costa-Lotufo et al., 2005). In this perspective, tests in *Drosophila melanogaster* are interesting and able to predict the therapeutic potential of different products. Indeed, due to the high similarity to mammalian genes, easy handling and maintenance, *D. melanogaster* is an organism test validated for the study of carcinogenicity, mutation and recombination (Baran, 2004). The Somatic Mutation and Recombination Test (SMART) is considered a cheap method capable of generating reliable and reproducible results (Graf et al., 1984; Spanó et al., 2001), besides being precise in discriminating simultaneously mutagenic, clastogenic, and/or recombinogenic agents. Moreover, it detects the genotoxicity of compounds of different chemical classes, complex mixtures, as well as aerial particles (Amaral, 2001; de Melo Reis et al., 2016; de Morais et al., 2016; Fernandes, 2017; Sarıkaya et al., 2016).

The test for detection of epithelial tumor (ETT) is widely used for the evaluation of carcinogenic or anticarcinogenic activity in an economic, fast, and sensitive way to different treatments. Different protocols are described including experimental designs with isolated or combined compounds, in strategies with pre-treatment, co-treatment and post-treatment (Lima et al., 2018; Nepomuceno, 2015; Orsolin et al., 2012; Vasconcelos et al., 2017).

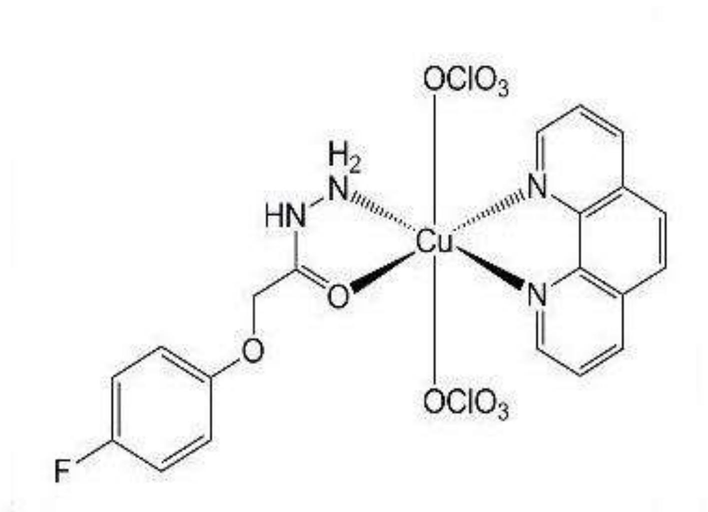
In the present study, these methodologies were performed aiming to assess the mutagenic/recombinogenic and antitumoral potentials of the DRI-12 in *D. melanogaster*. Considering the pharmacological and anticancer effect played by essential metals, like copper, we hypothesized that DRI-12 is a promising prototype in combating tumor cells.

## 2. MATERIAL AND METHODS

### *2.1 DRI-12 and Doxorubicin*

Paixão and collaborators synthesized, in 2017, the compound DRI-12 (Figure 1), a non-hygroscopic complex, stable in air and light and soluble in DMSO (Dimethylsulfoxide) and water. The compound was supplied by professor Dr. Wendell Guerra of the Chemistry Institute of the Federal University of Uberlandia and the serial dilutions (0.015; 0.031; 0.062; 0.125 e 0.250mM) were prepared using reverse osmosis water.

**Figure 1.** Representation of the chemical structure of metal complex.



**Source**: PAIXÃO et al., (2017).

Doxorubicin Hydrochloride (DXR), commercially known as Adriblastina® (CAS 25316-40-9, batch 5PL5111, registered, imported and distributed by the laboratory Pfizer, Sao Paulo), was used in the present study as a positive control at 0.4mM (diluted in autoclaved reverse osmosis water). DXR at 0.4mM was previously able to generate ROS and induce homologous recombination in *D. melanogaster* through topoisomerase inhibition (Lima et al., 2018; Machado et al., 2013; Orsolin et al., 2015; Silva-Oliveira et al., 2016).

*2.2 Toxicity test in Drosophila melanogaster*

*Drosophila*s used in this experiment were preserved and handled at LABCIM (Laboratory of Cytogenetic and Mutagenesi of the University Center of Patos de Minas - UNIPAM). The lineages of *D. melanogaster* were kept inside a B.O.D incubator at 25° C and 60% of humidity with a photoperiod of 12 hours. The toxicity test was performed to define the concentrations to be used in the ETT and SMART tests. Thirty heterozygous larvae *wts +/+ mwh,* from the cross between virgin females of *wts/TM3,* $Sb^1$ and males *mwh/mwh* were grown in medium containing the compound DRI-12 at concentrations of 0.015; 0.031; 0.062; 0.125; 0.250; 1.00; 2.00; 4.00 and 8.00mM.

According to Spanó et al. (2001) the larvae were exposed to the culture medium containing the compound DRI-12 and mashed potatoes (Yoki® Alimentos S.A), feeding for 48 hours (chronic treatment). At the end of the entire development phase, which lasts approximately one week, the emerging flies were preserved in 70% alcohol. Subsequently, the individuals were counted in a stereoscopic microscope. A survival curve was then constructed in order to establish the toxicity of the compound based on the percentage of flies surviving the treatment. It was also possible to determine the lethal dose of DRI-12 for *D. melanogaster*. Statistical significance ($p < 0.05$) was determined using the Chi-square test, by the GraphPad Prism 6.0 software (GraphPad Software Inc., La Jolla, California, USA).

*2.3 SMART test in D. melanogaster wings*

As established by (Graf and van Schaik, 1992) for the SMART test, the mutant lineages of *D. melanogaster* used were kindly supplied by Dr. Urich

Graf, of the Toxicology Institute, University of Zurich, Shwerzenbach, Switzerland. The test uses three lineages, *mwh*, *flr*$^3$ and *ORR*, which possess the genetic markers *multiple wing hairs* (*mwh*, 3-0,3) and *flare-3 (flr*$^3$, 3-38,8). Two crosses were performed, the *Standard* (ST) *Cross*, in which virgin females *flr*$^3$*/ In*(*3LR*)*TM , ri p*$^p$ *sepI(3)89Aa bx*$^{34e}$ and *Bd*$^s$ are crossed with males *mwh/mwh;* and the *High Bioactivation* (HB) *Cross*, in which virgin females *ORR; flr*$^3$*/ In*(*3LR)TM, ri p*$^p$ *sepI(3)89Aa bx*$^{34e}$ and *Bd*$^s$ are crossed with males *mwh/mwh*.

The crosses mentioned above produced two types of progeny: (i) trans-heterozygous individuals (MH) for the marker genes and (ii) balancer-heterozygous individuals (BH) (Andrade and Lehmann, 2003; Guzmán-Rincón and Graf, 1995). BH individuals are phenotypically differentiated from MH individuals by the presence of indentations on the edge of the wings, a characteristic conferred by the *TM3* marker, leaving them with a serrated aspect (Graf et al., 1984).

The experiment was conducted according to the protocol described by Graf et al. (1984). The collection of ORR and *flr*$^3$ virgin females was performed at 2-hours intervals, between 9 a.m and 5 p.m. After this stage, 100 females of each lineage (ORR and *flr3*) were placed together with 50 males (*mwh*) for the mentioned crossings. The eggs were laid for 8 hours in flasks containing a solid base of agar (4% agar in water) and a layer of yeast (*Sacharomyces cerevisae*) supplemented with sugar. After 72 hours of oviposition, third stage larvae were washed with reverse osmosis water and collected with the aid of a fine mesh steel sieve. Inside the fume hood, serial dilutions of the compound DRI-12 and DXR were performed.

Thereafter, chronic treatment was performed, in which the afore mentioned larvae were transferred to 25 mL flasks containing 5 mL of alternative medium (mashed potatoes) associated with DRI-12 complex in the concentrations of 0.015; 0.031; 0.062; 0.125 and 0.250mM. Reverse osmosis water was used as a negative control and DXR (0,4mM) as a positive control.

After this procedure, the flies fed on the medium and, a week later, completed the stages of development (metamorphosis). Then the adult individuals were collected and kept in 70% ethanol. Subsequently, the wings of the collected flies were detached using entomological forceps under a stereoscopic microscope and placed in pairs on histological slides, with 5 pairs of female wings at the top of the slide and 5 pairs of male wings at the bottom. The wings were fixed with Faure solution (50 mg of gum arabic, 30 g of chloral hydrate, 30 mL of glycerol, 50 mL of ultrapure water). The analysis of the wings was performed under a light microscope, at a magnification of 400x, recording the number, types, size and position of the spots. The trichomes present on the dorsal and ventral surface of the wings were observed in order to identify mutant hair spots classified as simple (*mwh* or *flr*$^3$) or twin (*mwh* and *flr*$^3$) and as small (1 to 2 mutant cells) or large (with more than 3 mutant cells). Sections (A, B, C, C', D, D' and E) were used to record the scale of each spot.

The statistical analysis of the experiment was carried out using the conditional binomial test of (Kastenbaum and Bowman, 1970), at a significance level of 5%. The procedure proposed by (Frei and Würgler, 1988) was used for the analysis of multiple decisions, generating four different diagnoses: positive, weak positive, negative or inconclusive.

Based on the frequency of induction of clones per $10^5$ cells, the recombinogenic activity was calculated as: Frequency of mutation (FM) = frequency of clones in BH flies/frequency of clones in MH flies. Frequency of recombination (FR) = 1 - FM. The percentage of inhibiton of DRI-12 was calculated using the frequency of clones per $10^5$ cells, corrected by control, according to the following formula: [DXR alone - (DRI -12 + DXR) / DXR alone] × 100 (Abraham, 1994).

*2.4 Epithelial Tumor Test (ETT) in Drosophila melanogaster*

ETT has been used to evaluate the carcinogenic or anticarcinogenic activity of different compounds/substances. This test allows the execution of simple and combined therapies, in co-treatment and post-treatment strategies. In co-treatment, the larvae are simultaneously exposed to DXR and the compound tested, whereas in the post-treatment, the larvae are previously induced to the tumor and, shortly after 6 hours, they are exposed to the substance under study, in order to verify the reversal of damages (Nepomuceno, 2015).

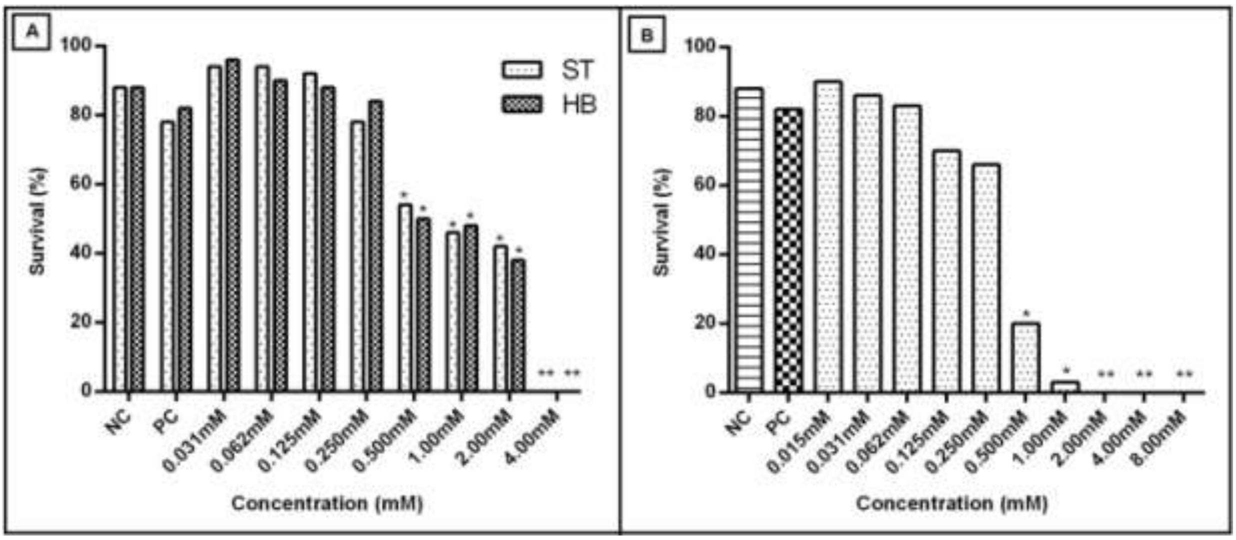
Two mutant lineages of *D. melanogaster* were used, including virgin females *wts/TM3, Sb1* and males *mwh/mwh*. The collection of virgin females *wts/TM3,* $Sb^1$ and males *mwh/mwh* was carried out for 3 consecutive days, in flasks containing standard culture medium. On the last day, the two lineages were placed together for crossing. About 48h after this period, males and females were placed in flasks containing a culture medium appropriate for laying (yeast and sugar), where the females laid their eggs. The 72h larvae were fed with culture medium containing the DRI-12 copper complex at the concentrations of 0.015; 0.031; 0.062; 0.125 and 0.250mM (in quadruplicate), chosen based on the toxicity test result. The assay was performed with DRI-12 alone or combined with DXR (0.4mM). The entire procedure was carried out under aseptic and controlled conditions. The reverse osmosis water was used as a negative control and DXR (0.4mM) as a positive control. Adult flies were collected and kept in ethanol ($C_2H_6O$) 70%.

The entire body of adult flies was analyzed under a stereoscopic microscope with the aid of entomological forceps, excavated plaque and glycerin. The identification and selection of the individuals used in the analyzes were based on the characteristic of the body and head hairs of the flies. Adult flies that had the chromosomal balancer (*TM3*, *Sb*$^{1}$) carriers the *wts+/+TM3* genotype, which have a short and thick hair phenotype, were discarded. Individuals with long and thin hairs, and genotype (*wts* +/+ *mwh*), were analyzed due to the presence of the gene under study (*wts*), homologous to *LATS1 (Large tumor suppressor kinase* 1) tumor suppressor in humans (Siam et al., 2009). The statistical analysis of the ETT test was performed using the Mann-Whitney test, by the Prophet software, at a significance level of 5%.

## 3. RESULTS

DRI-12, as a promising metallic compound in the treatment of neoplasms, was evaluated for its mutagenic/recombinogenic and anticarcinogenic potential in *D. melanogaster*. For this purpose, the Toxicity Test (TX) was initially performed with larvae from the SMART and ETT tests in order to define the lethal dose (DL) and the concentrations of the compound to be used later (Figure 2). Concentrations that did not show significant toxicity (0.015; 0.031; 0.062; 0.125 and 0.250mM) were used in the subsequent assays. When there was a significant decrease (p <0.05) in the percentage of larval survival, it was concluded that the compound presented a toxicity that interferes with the development of the larvae. Furthermore, the number of emerging adults needs to be enough to conduct the next stages of the experiment (Demir et al., 2013). In the present study, from the 4.00mM concentration of DRI-12, no *D. melanogaster* emerged in the SMART assay (Fig. 2 A), which indicates that this was the DL for the lineage used in the experiment. For individuals used in the ETT test, the DL was 2.00mM (Fig. 2 B).

**Figure 2.** Survival rate of *Drosophila melanogaster* obtained from the Toxicity test (TX) of different concentrations of the compound DRI-12. **(A)** lineages of the Standart (ST) Cross and High Bioactivation (HB) Cross used in the SMART test; **(B)** lineages used in the ETT test.




Tables 1 and 2 show the results for the SMART test, with the frequencies of mutant spots: small single (MSP), large single (MSG), twin (MG) and the total of spots of the marked trans-heterozygous individuals from the standard cross and the high bioactivation cross. Table 1 shows the results of the two crosses of individuals treated with DRI-12 at concentrations of 0.015; 0.312; 0.062; 0.125 and 0.250mM, the positive control (DXR - 0.4mM) and the negative control (reverse osmosis water). In none of the tested concentrations, both in descendants of the ST and HB crosses, there were significant differences in the total frequency of spots compared to the negative control.

**Table 1.** Results obtained in the marked trans-heterozygous (MH) descendants of *Drosophila melanogaster* derived from the standard (ST) cross and high bioactivation (HB) crosss treated with different DRI-12 concentratios, negative control (reverse osmosis water) and positive control (0.4mM Doxorrubicin).

| Treatments | | | Spots per fly ($N^0$ of spots); statistical diagnosis[a] | | | | | | | | | | | | Spots with *mwh* clone[c] ( *n* ) | Frequency of clone formation/ $10^5$ cells per cell division [d] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DXR (mM) | DRI 12 (mM) | **Number of flies (*N*)** | Small single (1-2 cels)[b] *m* = 2 | | | Large single (>2 cels)[b] *m* = 5 | | | Twin *m* = 5 | | | Total spots *m* = 2 | | | | Observed | Control corrected |
| *mwh/flr[3]* | | | | | | | | | | | | | | | | | |
| *ST Cross* | | | | | | | | | | | | | | | | | |
| 0 | 0 | 60 | 0.40 | (24) | | 0.03 | (2) | | 0.02 | (1) | | 0.45 | (27) | | 27 | 0.92 | |
| 0.4 | 0 | 60 | 2.62 | (157) | + | 4.52 | (271) | + | 5.33 | (320) | + | 12.47 | (748) | + | 697 | 23.80 | 22.80 |
| 0 | 0.015 | 60 | 0.32 | (19) | - | 0.03 | (2) | I | 0.08 | (5) | i | 0.43 | (26) | - | 23 | 0.79 | - 0.14 |
| 0 | 0.031 | 60 | 0.35 | (21) | - | 0.05 | (3) | I | 0.02 | (1) | i | 0.42 | (25) | - | 23 | 0.79 | - 0.14 |
| 0 | 0.062 | 60 | 0.35 | (21) | - | 0.03 | (2) | I | 0.03 | (2) | i | 0.42 | (25) | - | 25 | 0.85 | - 0.07 |
| 0 | 0.125 | 60 | 0.23 | (14) | - | 0.05 | (3) | I | 0.12 | (7) | i | 0.40 | (24) | - | 21 | 0.72 | - 0.20 |
| 0 | 0.250 | 60 | 0.25 | (15) | - | 0.00 | (0) | I | 0.12 | (7) | i | 0.37 | (22) | - | 16 | 0.55 | - 0.38 |
| *HB Cross* | | | | | | | | | | | | | | | | | |
| 0 | 0 | 60 | 0.62 | (37) | | 0.20 | (12) | | 0.00 | (0) | | 0.82 | (49) | | 48 | 1.64 | |
| 0.4 | 0 | 60 | 1.30 | (78) | + | 1.,10 | (606) | | 1.98 | (119) | + | 13.38 | (803) | + | 782 | 26.71 | 25.07 |
| 0 | 0.015 | 60 | 0.27 | (16) | - | 0.13 | (8) | - | 0.03 | (2) | i | 0.43 | (26) | - | 26 | 0.89 | - 0.75 |
| 0 | 0.031 | 60 | 0.30 | (18) | - | 0.12 | (7) | - | 0.02 | (1) | i | 0.43 | (26) | - | 26 | 0.89 | - 0.75 |
| 0 | 0.062 | 60 | 0.38 | (23) | - | 0.03 | (2) | I | 0.00 | (0) | i | 0.42 | (25) | - | 25 | 0.85 | - 0.89 |
| 0 | 0.125 | 60 | 0.22 | (13) | - | 0.10 | (6) | - | 0.07 | (4) | i | 0.38 | (23) | - | 23 | 0.79 | - 0.85 |
| 0 | 0.250 | 60 | 0.10 | (6) | - | 0.10 | (6) | - | 0.00 | (0) | i | 0.20 | (12) | - | 12 | 0.41 | - 1.23 |

Marker-trans-heterozygous flies (*mwh*/*flr3*) were evaluated.
a Statistical diagnosis according to Frei and Würgler (1988, 1995): +, positive; -, negative; i, inconclusive. m = multiplication factor for significantly negative results. Level of significance $p \leq 0.05$.
b Including rare single flr3 spots.
c Considering the *mwh* clones for the single spots and *mwh* for the twin spots.
d Frequency of clone formation: clones/flies/48,800 cells (without size correction) (Frei et al., 1992).
e Only *mwh* single spots can be observed in heterozygous individuals *mwh*/*TM3*.

The results of the treatment of larvae of DRI-12 associated with DXR (0.4mM) are presented in Table 2. A reduction in the frequency of spots was observed in ST (0.015, 0.031, 0.125 and 0.250mM) and HB (0.125 and 0.25mM) descendants, when compared to the positive control. DXR can directly affect the cell membrane through interaction with plasma proteins leading to its enzymatic reduction. This event can lead to the formation of ROS, such as the hydroxyl radical. Although this mechanism makes DXR a potent anticancer, it is responsible for related side effects (Tacar et al., 2013).

**Table 2.** Results obtained in the marked trans-heterozygous descendants (MH) and balancer-heterozygous (BH) of *Drosophila melanogaster* derived from the DRI-12 associated with Doxorubicin (0.4mM) and negative control (reverse osmosis water).

| Treatments: DXR (mM) | Treatments: DRI 12 (mM) | Number of flies (*N*) | Spots per fly ($N^0$ of spots); statistical diagnosis[a]: Small single (1-2 cels)[b] $m$ = 2 | | | Large single (>2 cels)[b] $m$ = 5 | | | Twin $m$ = 5 | | | Total spots $m$ = 2 | | | Spots with *mwh* clone[c] ( *n* ) | Frequency of clone formation/ $10^5$ cells per cell division [d]: Observed | Control Corrected | Recombination | Inhibition (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***mwh/flr$^3$*** | | | | | | | | | | | | | | | | | | | |
| *Cruzamento ST* | | | | | | | | | | | | | | | | | | | |
| 0 | 0 | 60 | 0.40 | (24) | | 0.03 | (2) | | 0.02 | (1) | | 0.45 | (27) | | 27 | 0.92 | | | |
| 0.4 | 0 | 60 | 2.62 | (157) | + | 4.52 | (271) | + | 5.33 | (320) | + | 12.47 | (748) | + | 697 | 23.80 | 22.88 | 98.82 | |
| 0.4 | 0.015 | 60 | 1.40 | (84) | + | 4.52 | (271) | - | 5.05 | (303) | - | 10.97 | (658) | f+ | 596 | 20.36 | 19.43 | 98.37 | 15.08 |
| 0.4 | 0.031 | 60 | 1.75 | (105) | f+ | 3.92 | (235) | - | 5.27 | (316) | - | 10.93 | (656) | f+ | 624 | 21.31 | 20.39 | 97.84 | 10.88 |
| 0.4 | 0.062 | 60 | 1.78 | (107) | f+ | 4.13 | (248) | + | 6.92 | (415) | f+ | 12.83 | (770) | - | 755 | 25.79 | 24.86 | | |
| 0.4 | 0.125 | 60 | 2.20 | (132) | - | 2.68 | (161) | f+ | 3.12 | (187) | + | 8.00 | (480) | f+ | 461 | 15.74 | 14.82 | 98.85 | 35.23 |
| 0.4 | 0.250 | 60 | 1.18 | (71) | + | 3.00 | (180) | f+ | 5.08 | (305) | - | 9.27 | (556) | f+ | 545 | 18.61 | 17.69 | 99.83 | 22.68 |
| *Cruzamento HB* | | | | | | | | | | | | | | | | | | | |
| 0 | 0 | 60 | 0.62 | (37) | | 0.20 | (12) | | 0.00 | (0) | | 0.80 | (48) | | 48 | 1.64 | | | |
| 0.4 | 0 | 60 | 1.30 | (78) | + | 10.1 | (606) | + | 1.98 | (119) | + | 13.38 | (803) | + | 782 | 26.71 | 25.07 | 98.48 | |
| 0.4 | 0.015 | 60 | 1.43 | (86) | - | 9.28 | (557) | - | 2.50 | (150) | f+ | 13.22 | (793) | - | 781 | 26.67 | 25.03 | | |
| 0.4 | 0.031 | 60 | 1.85 | (111) | f+ | 9.22 | (553) | - | 2.53 | (152) | f+ | 13.60 | (816) | - | 806 | 27.53 | 25.89 | | |
| 0.4 | 0.062 | 60 | 0.58 | (35) | + | 10.3 | (619) | - | 3.22 | (193) | f+ | 14.13 | (848) | - | 847 | 28.89 | 27.25 | | |
| 0.4 | 0.125 | 60 | 2.07 | (124) | + | 5.45 | (327) | + | 0.95 | (57) | + | 8.47 | (508) | f+ | 498 | 17.01 | 15.37 | 98.70 | 38.69 |
| 0.4 | 0.250 | 60 | 1.93 | (116) | f+ | 7.70 | (462) | f+ | 2.08 | (125) | - | 11.72 | (703) | f+ | 695 | 23.74 | 22.10 | 99.23 | 11.85 |
| ***mwh/TM3*** | | | | | | | | | | | | | | | | | | | |
| *Cruzamento ST* | | | | | | | | | | | | | | | | | | | |
| 0 | 0 | 60 | 0.15 | (9) | | 0.02 | (1) | | | | | 0.17 | (10) | | 10 | 0.34 | | | |
| 0.4 | 0 | 60 | 0.20 | (12) | i | 0.10 | (6) | i | | | | 0.30 | (18) | i | 18 | 0.61 | 0.27 | | |
| 0.4 | 0.015 | 60 | 0.13 | (8) | i | 0.13 | (8) | i | | | | 0.27 | (16) | i | 16 | 0.55 | 0.20 | | |
| 0.4 | 0.031 | 60 | 0.25 | (15) | i | 0.13 | (8) | i | | | | 0.38 | (23) | i | 23 | 0.79 | 0.44 | | |
| 0.4 | 0.125 | 60 | 0.13 | (8) | i | 0.12 | (7) | i | | | | 0.25 | (15) | i | 15 | 0.51 | 0.17 | | |
| 0.4 | 0.250 | 60 | 0.15 | (9) | i | 0.03 | (2) | i | | | | 0.18 | (11) | i | 11 | 0.38 | 0.03 | | |
| *Cruzamento HB* | | | | | | | | | | | | | | | | | | | |
| 0 | 0 | 60 | 0.13 | (8) | | 0.02 | (1) | | | | | 0.15 | (9) | | 9 | 0.31 | | | |
| 0.4 | 0 | 60 | 0.28 | (17) | i | 0.05 | (3) | i | | | | 0.33 | (20) | + | 20 | 0.68 | 0.38 | | |
| 0.4 | 0.125 | 60 | 0.17 | (10) | i | 0.08 | (5) | i | | | | 0.25 | (15) | i | 15 | 0.51 | 0.20 | | |
| 0.4 | 0.250 | 60 | 0.10 | (06) | i | 0.13 | (8) | i | | | | 0.23 | (14) | i | 14 | 0.48 | 0.17 | | |

Marker-trans-heterozygous flies (*mwh/flr3*) and balancer-heterozygous flies (*mwh/TM3*) were evaluated.
[a] Statistical diagnosis according to Frei and Würgler (1988, 1995): +, positive; -, negative; i, inconclusive; f+, fracamente positivo. $m$ = multiplication factor for significantly negative results. Level of significance $p \leq 0.05$.
[b] Including rare single *flr3* spots.
[c] Considering the *mwh* clones for the single spots and *mwh* for the twin spots.
[d] Frequency of clone formation: clones/flies/48,800 cells (without size correction) Frei et al. (1992).
[e] Calculated as {[DXR alone – DXR + DRI-12] /DXR} x 100, according to Abraham (1994)
[f] Only *mwh* single spots can be observed in heterozygous individuals *mwh/TM3*, since the balancer chromosome *TM3* does not contain the mutant gene *flr3.*

Considering that there was a reduction in the frequency of spots in both the ST and HB crosses, BH descendants from both crosses were analyzed to quantify the percentage of recombinogenic events and whether the reduction effect was predominantly in spots derived from mutation or recombination. In the ST cross, the results showed that the major event in the formation of spots was recombination, with percentages of 98.82; 98.97; 97.84; 98.85 and 99.83% at concentrations of 0.015; 0.031; 0.125 and 0.250mM, respectively. In the HB cross, the results revealed that 98.70 and 99.23% of the frequencies were recombination at concentrations of 0.125 and 0.250mM, respectively. Regarding the percentage of reduction of mutant spots, in the ST cross the inhibition was 15.08, 10.88, 35.23 and 22.68% at concentrations of 0.015, 0.031, 0.125 and 0.250mM, respectively. Otherwise, in the HB cross this reduction was 38.69 and 11.85% at concentrations of 0.125 e 0.250mM, respectively.

Results for ETT are presented in Table 3, containing co-treatment results, shows the frequencies of tumors in the different body segments of *D. melanogaster* in individuals treated with positive control (DXR 0.4mM), negative control (reverse osmosis water) and with different concentrations of DRI-12 (0.015, 0.031, 0.062, 0.125, and 0.250mM) isolated and associated with DXR (0.4mM). Larvae exposed only to treatment with DRI-12 at concentrations of 0.015; 0.031; 0.062; 0.125 and 0.250mM presented frequencies of 0.41; 0.24; 0.23; 0.22 and 0.18 tumors per fly, respectively. None of the tumor frequencies obtained in the treatment with DRI-12 showed statistically difference from the negative control, according to the Mann-Whitney Test ($p < 0.05$). This result shows the absence of a carcinogenic effect of DRI-12 at the tested concentrations. Subsequent malignant neoplasms (SMNs) are known to be one of the most serious and potentially lethal complications of cancer and its therapy. Chemotherapy with platinum analogues has a significant association with the risk of

SMN (Fung and Travis, 2018), because when they bind to DNA, they cause the molecule to twist, inhibiting transcription and causing the death of tumor cells. Such activity could be suggested in the effects performed by DRI-12 in *D. melanogaster*.

**Table 3.** Tumor clone frequency observed in the co-treatment in *Drosophila melanogaster*, heterozygote for the *wts* tumor suppressor gene, treated with doxorrubicin and different concentrations of DRI -12.

| Treatments | | | Number of tumors analyzed | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DRI 12 (mM) | DXR (mM) | Number of flies | Eye | Head | Wing | Body | Leg | Halter | Total | Frequency/ Number of tumors per fly | Tumor reduction (%) |
| 0 | 0 | 200 | 2 | 4 | 13 | 38 | 2 | 1 | 59 | 0.29 | |
| 0.015 | 0 | 200 | 3 | 12 | 27 | 38 | 1 | 2 | 83 | 0.41 | |
| 0.031 | 0 | 200 | 2 | 6 | 3 | 32 | 5 | 0 | 48 | 0.24 | |
| 0.062 | 0 | 200 | 2 | 8 | 17 | 15 | 3 | 2 | 47 | 0.23 | |
| 0.125 | 0 | 200 | 2 | 5 | 13 | 21 | 2 | 1 | 44 | 0.22 | |
| 0.250 | 0 | 200 | 2 | 4 | 10 | 17 | 2 | 2 | 37 | 0.18 | |
| 0 | 0.4 | 200 | 23 | 30 | 197 | 372 | 49 | 12 | 675 | 3.37* | |
| 0.015 | 0.4 | 200 | 4 | 18 | 64 | 145 | 24 | 1 | 239 | 1.19** | 64.69 |
| 0.031 | 0.4 | 200 | 6 | 8 | 11 | 91 | 3 | 0 | 115 | 0.57** | 83.09 |
| 0.062 | 0.4 | 200 | 3 | 6 | 36 | 57 | 7 | 0 | 106 | 0.53** | 84.28 |
| 0.125 | 0.4 | 200 | 3 | 12 | 15 | 32 | 11 | 7 | 84 | 0.42** | 87.54 |
| 0.250 | 0.4 | 200 | 6 | 5 | 6 | 15 | 7 | 2 | 41 | 0.20** | 94.07 |

Statistical diagnosis according to the Mann-Whitney Test. Level of significance $p \leq 0.05$
* Value considered different from the negative control ($p \leq 0.05$).
** Value considered different from the positive control (DXR 0.4 mM) ($p \leq 0.05$). DXR, doxorubicin.

For the negative control (reverse osmosis water), a frequency of 0.29 tumors per fly was observed, probably due to the occurrence of spontaneous mutations in the test organism. The value observed in the negative control was significantly different from that observed in the positive control (DXR 0.4mM), which was 3.37 tumors per fly. This shows that the lineage responds to tumor

induction. The results obtained are compatible with previous studies (Lima et al., 2018; Orsolin et al., 2012; Vasconcelos et al., 2017).

When evaluating the anticarcinogenic action in the co-treatment (simultaneous exposure of DRI-12 associated with 0.4mM DXR), a significant difference was observed at the five concentrations tested (0.015; 0.031; 0.0625; 0.125 and 0.250mM) in relation to the positive control (0.4mM DXR) according to the Mann-Whitney test ($p < 0.05$). The alone treatment showed total frequencies of tumors per fly of 1.19; 0.57; 0.53; 0.42 and 0.20, respectively. It is clearly observed that as the concentration of the copper complex increases, there was a reduction in the frequency of tumors, reaching levels comparable to the negative control in the highest concentrations of DRI-12, which shows the modulating effect of the complex (reduction of tumors of 64.69; 83.09; 84.28; 87.54 and 94.07%, respectively). In this context, it is suggested that DRI-12 has modulated the action of DXR and redox mechanisms are also associated, leading to increased damage to cells mutated by DXR, and consequently, cell apoptosis.

The post-treatment assay in *D. melanogaster*, was carried out under the same experimental conditions as the co-treatment, however, exposing the larvae to DXR first. After 6 hours, the flies were subjected to DRI-12. The results are shown in table 4.

**Table 4.** Tumor clone frequency observed in the post-treatment in *Drosophila melanogaster*, heterozygote for the *wts* tumor suppressor gene, post-treated with doxorrubicin and different concentrations of DRI -12.

| Treatments | | | Number of tumors analyzed | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **DRI 12 (mM)** | **DXR (mM)** | **Number of flies** | **Eye** | **Head** | **Wing** | **Body** | **Leg** | **Halter** | **Total** | **Frequency/ Number of tumors per fly** | **Tumor reduction (%)** |
| 0 | 0 | 200 | 0 | 1 | 10 | 47 | 7 | 1 | 64 | 0.32 | |
| 0 | 0.4 | 200 | 5 | 34 | 139 | 392 | 64 | 1 | 634 | 3.17 * | |
| 0.015 | 0.4 | 200 | 0 | 5 | 39 | 142 | 5 | 0 | 191 | 0.95 ** | 70.03 |
| 0.031 | 0.4 | 200 | 0 | 9 | 37 | 99 | 9 | 4 | 158 | 0.79 ** | 75.08 |
| 0.062 | 0.4 | 200 | 0 | 9 | 45 | 78 | 10 | 1 | 142 | 0.71 ** | 77.61 |
| 0.125 | 0.4 | 200 | 0 | 2 | 59 | 62 | 8 | 2 | 133 | 0.66 ** | 79.18 |
| 0.250 | 0.4 | 200 | 1 | 5 | 32 | 60 | 7 | 0 | 104 | 0.52 ** | 83.60 |

Statistical diagnosis according to the Mann-Whitney Test. Level of significance $p \leq 0.05$
* Value considered different from the negative control ($p \leq 0.05$).
** Value considered different from the positive control (DXR 0.4 mM) ($p \leq 0.05$).
DXR, doxorubicin.

A significant difference in tumor frequencies was observed at all concentrations tested (0.015; 0.031; 0.062; 0.125 and 0.250mM) in relation to the positive control (DXR 0.4mM) according to the Mann-Whitney test (p<0,05). The total frequencies of tumors obtained per fly were: 0.95; 0.79; 0.71; 0.66 and 0.52, respectively. It is noted that, with the increase in the concentration of the copper complex, there was a reduction in the frequency of tumors, which shows the anticarcinogenic potential of the complex and its effect in reducing damage (reduction of tumors of 70.03; 75.08; 77.61; 79.18 and 83.60%, respectively). In this context, the results obtained suggest a satisfactory antitumor response of DRI-12, which needs to be validated in other animal models.

## 4. DISCUSSION

These data suggest that the progeny used in ETT was more sensitive to the toxic effect of DRI-12 than individuals used in SMART. Similar results were obtained by (Naves, 2017), in which flies in the ETT test were more sensitive to toxicity to titanium dioxide nanocrystals ($TiO2$) than SMART progenies. Otherwise, in both tests, when compared to the negative control, treatments with concentrations above 0.500mM significantly compromised the survival of the flies. Therefore, these doses were not used in the next experiments. In toxicity studies, *in vivo* tests may simulate what happens systematically. Indeed, to avoid overuse of mammals, *Drosophila* is a validated model for toxicological assays, since about 80% of the genes associated with human diseases have homologues in these individuals (Koh et al., 2006; Marcos and Carmona, 2013).

According to our results, DRI-12 possibly modulated the action of DXR leading to a reduction in the frequencies of mutant spots, when compared to the positive control (DXR 0.4mM). It is known that the main mechanism of action of DXR is by inhibiting topoisomerases (Tacar et al., 2013). According to (Ceramella et al., 2019) this is desirable for antitumoral compounds. Topoisomerases are vital enzymes in cell proliferation and targeting them causes DNA damage and ultimately, cell death. Previously studies demonstrated the action of some copper (II) metallic complexes through inhibition of Topoisomerase I causing DNA double strand break (DSBs) (Arjmand et al., 2012; Tabassum et al., 2012; Vutey et al., 2016). Thus, it can be inferred that DRI-12 may have potentiated the damage induced by DXR in *D. melanogaster* cells, activating apoptosis and preventing the expression of the mutant phenotype.

Our results indicated the absence of a mutagenic/recombinogenic effect of DRI-12 in somatic cells of *D. melanogaster*, suggesting a possible selectivity of DRI-12. González et al. (2013) emphasized that some copper complexes may effectively kill cancer cells without showing mutagenic activity (González et al., 2013). In fact ideal drugs for cancer treatment should not damage normal cells, but it is necessary that, at the same time, make tumor cells unviable (Cadavid-Vargas et al., 2019). DRI-12 meets those criteria, which makes it promised an antitumor compound.

Considering the results for SMART test in Tables 1 and 2, DXR, our positive control, was predominantly recombinogenic, with 97.44% in ST and 97.45% in HB. This result is in line with other scientific findings (Andrade and Lehmann, 2003; Lima et al., 2018; Machado et al., 2013; Oliveira et al., 2017; Orsolin et al., 2015; Silva-Oliveira et al., 2016). DXR is a potent and effective chemotherapeutic agent used to treat various types of cancers, such as breast, lung, bladder, thyroid, ovary, bone and soft tissue sarcomas, Hodgkin's and non-Hodgkin lymphomas, neuroblastoma, Wilms' tumor and leukemias (Garnis et al., 2004). However, DXR causes serious cardiac toxicity and therapeutic resistance (Tallaj et al., 2005). It is an anthracycline antineoplastic antibiotic isolated from cultures of *Streptomyces peucetius*, which produces its effects mainly through direct action on DNA (Malla et al., 2010), inducing DSBs. In human cells, DSBs are repaired by homologous recombination pathways (Poplawski et al., 2010), and this mechanism was observed in the numbers referring to our positive control.

Although DRI-12 decreased the expression of mutant spots in both crosses, the recombinogenic profile of DXR was not changed. One of the mechanisms related to tumor resistance to DXR is the expression of the P glycoprotein, which mediates the efflux of the drug and its trapping within lysosomes (Germann, 1996;

Gottesman et al., 2002; Yamagishi et al., 2013). We suggest that DRI-12 may have enhanced the effect of DXR, increasing its release from lysosomes. Seebacher el tal (2016) also demonstrated a strategy to relocate of stored DXR from lysosomes through treatment of breast tumor cells with copper metallic complexes (Seebacher et al., 2016). In their study, they observed the synergism between DXR and cooper complexes, which increased the instability of the lysosome membrane, releasing DXR. Thus, the reduction in the frequency of spots observed in the present study, both in the ST and HB crosses, may be explained by synergism between DXR and DRI-12, inducing cell death. Copper compounds are known to have a broad spectrum of activities and low toxicity, being able to overcome the resistance of platinum analogues (Ahmad et al., 2018).

Copper complexes associated with phenanthroline induce oxidative damage by intercalation with the minor groove of the DNA molecule, mainly generating the hydroxyl radical (Molphy et al., 2015; Tisato et al., 2010). In a study conducted by Lopes et al. (2018), using a ternary copper complex, it was also suggested that the recombinogenic activity was high enough to induce apoptosis, thus preventing the detection of mutant cells in the wings of *D. melanogaster*. These results are supported by the toxicity test result, because although the compound was not lethal at the concentrations tested, toxicity increased at the highest concentrations, which may be explained by the induction of cell death due to excessive damage. In this context, the results obtained in this research are also explained by the excess of ROS within the cells (Alvarez et al., 2010).

Copper-based compounds increase can cause the depletion of the activity of the enzyme glutathione peroxidase (GSH) which regulates the concentrations of ROS and cause the conformational change in the active site of the enzyme glutathione S-transferase. Knowing that these enzymes are fundamental in the cellular redox balance, the inhibition of their activities triggers a greater accumulation of ROS within the cells, and consequently oxidative stress and damage to biomolecules (Zafar et al., 2017), such as lipids, proteins and nucleic acids (Sissi et al., 2005). When there are excessive levels of ROS, intracellular oxidative stress is further increased, causing a direct response of cell death by apoptosis (Huffman and O'Halloran, 2001). This behavior happens through the release of pro-apoptotic factors by the mitochondria when exposed to exacerbated levels of ROS (Fruehauf and Meyskens, 2007). High amounts of copper can lead to this scenario and, therefore, chemotherapeutics which carry this metal have been explored in cancer treatment (Chen and Sigman, 1986; Chikira et al., 2015; Costa et al., 2011; Ruiz-Azuara and E Bravo-Gomez, 2010). In this context, the combination of redox-active molecules with conventional therapy can be useful to enhance treatments and overcome resistance mechanisms.

Results obtained by (Silva et al., 2014) with [Cu (hyd) (phen) (ACN) (ClO4)] (ClO4), a ternary copper (II) complex, structurally similar to DRI-12, containing the hydrazide of 2-furoic acid, demonstrated its action against a leukemia cell line. Complexes in this format have been demonstrated to penetrate cells and inhibit cell growth in a concentration-dependent manner (Paixão et al., 2017). Our group also evaluated other copper (II) complex similar to that of the present study in tumor and normal murine cells lines and the compound was effective and selective. There was also an increase in the production of ROS, autophagic dysfunction and induction of apoptosis (Polloni et al., 2019). However, there are still no studies that report the possible antitumoral effect, *in vivo*, of the compound DRI-12. The present results are pioneers in demonstrating the antitumoral of DRI-12 in *D. melanogaster*. *D.*

*melanogaster* is an organism capable to activate, enzymatically, promutagens and procarcinogens *in vivo* (Nepomuceno, 2015). According to Ahmad and contributors (2018), copper (II) complexes have potentially effective anticancer activity *in vivo* and drugs should be evaluated for their ability to generate tumors reduce tumors. For this purpose, we conducted ETT test in co-treatment (DRI-12+DXR) and post-treatment assays and we demonstrated the anticarcinogenic and DXR modulator potential of DRI-12.

The use of copper complexes as chemotherapeutic agents has been highlighted, especially those with N, N-donors. Such complexes act as synthetic nucleases, facilitating intercalation with DNA. The [Cu (phen) 2]$^+$ complex, for example binds to the DNA minor groove and in combination with molecular oxygen induces chain scission by oxidation of the ribose skeleton, inhibiting the growth of tumor cells (Zhang et al., 2012).

Metal complexes containing the 1,10-phenanthroline ligand may be antitumor agents, as they increase the influx of neoplastic cells, inducing the apoptosis process by inhibiting proteasome activity and increasing DNA cleavage. In this context, copper complexes have been synthesized and evaluated for their biological potential showing promising results in inhibiting the growth of tumor cells (Chikira et al., 2015).

Previous studies have indicated that the components of DRI-12 prevented tumor cells from progressing the cell cycle, by signaling apoptosis (Lamsonl and Brignall, 1999; Truong-Tran et al., 2000). This is a biological mechanism capable of removing superfluous, mutant or moderately damaged cells. It is a cell death response to toxic agents and its deregulation is crucial in the mechanism of several pathologies, including cancer (Burgess, 2013; Su et al., 2015; Tallaj et al., 2005).

## 5. CONCLUSION

The copper (II) ternary complex, DRI-12, did not induce mutagenicity/ recombinogenicity and carcinogenicity. Additionally, DRI-12 presented anticarcinogenic potential and DXR modulator, desirable characteristics for a chemotherapeutic agent. Therefore, we suggest DRI-12 as a promising antitumoral agent. This compound should be widely studied in other tumor types and experimental models to better elucidate its mechanism of action, antitumoral effect, safety and selectivity.

**Highlights**

**The new copper complex has antineoplastic potential.**

**The new compound DRI 12 is a promising compound for cancer treatment.**

**Compound DRI 12 reduces blemish formation and does not generate toxic metabolites in healthy cells.**

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Divide the article into clearly defined sections.

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