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MOLECULAR

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**EFEITOS DOS COMPOSTOS QUERCETINA, QUERCETINA EM  
NANOEMULSÃO, RESVERATROL E RUTINA SOBRE A  
HEPATOTOXICIDADE E NEUROTOXICIDADE INDUZIDAS POR  
OXALIPLATINA EM CAMUNDONGOS**

PORTO ALEGRE, 2013

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para obtenção do Título de Mestre pelo  
Programa de Pós-Graduação em Biologia Celular  
e Molecular, Faculdade de Biociências, Pontifícia  
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*Dedico este trabalho a minha família, por todo amor e dedicação para comigo, por terem me apoiado em todas as minhas escolhas e, principalmente na concretização deste sonho. Ao meu marido, pelo carinho dispensado em todos os momentos que precisei. Muito obrigada por estarem do meu lado!*

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## RESUMO

**Introdução:** A oxaliplatina é um agente antineoplásico e tem sido amplamente utilizado no tratamento de vários tumores. É um composto derivado da platina de terceira geração desenvolvido com o propósito de ultrapassar as limitações da toxicidade, a resistência do tumor e a fraca biodisponibilidade oral associada à administração de cisplatina. Sintomas de neurotoxicidade estão associados ao uso da oxaliplatina e não existe tratamento adequado. Doses crescentes de oxaliplatina podem levar ao desenvolvimento de sensibilidade a frio e neuropatia sensorial periférica, e a um aumento da intensidade dos sintomas. Além disso, apesar da sua utilidade, a quimioterapia aumenta a taxa de desenvolvimento de danos hepáticos com atividade inflamatória. Esta manifestação pode ser chamada de esteato-hepatite associada à quimioterapia (CASH), uma forma mais grave da doença hepática gordurosa não alcoólica. Deste modo, o presente estudo, objetivou comparar o efeito de alguns compostos antioxidantes no desenvolvimento simultâneo da hepato e neurotoxicidade induzida pela oxaliplatina em camundongos. **Métodos:** Os camundongos Balb/c foram tratados intraperitonealmente (i.p.), com doses de 10 mg/kg de oxaliplatina durante 6 semanas, resultando em anodinia mecânica, e indução de esteatose hepática. Os compostos rutina (RUT) (20 mg/Kg/d), resveratrol (RSV) (100 mg/Kg/dia), quercetina (QT) (20 mg/Kg/dia) e nanoquercetina (NQT) (20 mg/Kg/dia), foram administrados diariamente por gavagem aos camundongos. *N*-acetilcisteína foi utilizada como controle. A eutanásia ocorreu no 43º dia após o tratamento. Foram analisadas a nocicepção mecânica, as aminotransferases ALT/AST, foi realizada avaliação histopatológica e da atividade da mieloperoxidase. A análise estatística foi realizada por ANOVA de uma via seguida do teste de Bonferroni. **Resultados:** Os tratamentos com resveratrol, rutina ou quercetina em nanoemulsão foram capazes de impedir a alodinia mecânica, quando comparados com o grupo tratado com oxaliplatina. Em relação ao efeito sobre a esteato-hepatite, os tratamentos com resveratrol, quercetina e quercetina em nanoemulsão reverteram significativamente o aumento de peso médio do fígado induzido pela oxaliplatina. Corroborando com estes dados, a avaliação histológica mostrou uma atenuação em todas as características de esteatose hepática

avaliados nos grupos tratados com resveratrol, rutina, quercetina e quercetina em nanoemulsão, apresentando características semelhantes ao controle positivo com *N*-acetilcisteína. Por outro lado, apenas os tratamentos com quercetina e quercetina em nanoemulsão foram capazes de reduzir a migração de neutrófilos medida pela atividade de mieloperoxidase. **Conclusão:** Os resultados sugerem que o uso dos compostos resveratrol, rutina, quercetina e quercetina em nanoemulsão podem se alternativas efetivas para o tratamento da hepato e neurotoxicidade induzida pela oxaliplatina no modelo testado.

**Palavras-chave:** esteato-hepatite, neuropatia, compostos fenólicos, oxaliplatina.

## ABSTRACT

**Introduction:** Oxaliplatin is an antineoplastic agent widely used in the treatment of some tumors. It is a third-generation platinum compound developed with the purpose of overcoming the limitations of toxicity, tumor resistance and poor oral bioavailability associated to cisplatin administration. Oxaliplatin-associated neurotoxicity represents the main dose limiting and there is not suitable treatment. Increasing doses of oxaliplatin can lead to the development of mechanical allodynia, cold sensitivity and peripheral sensory neuropathy, with increase of symptoms. Furthermore, despite its usefulness, chemotherapy with oxaliplatin increases the rate of developing hepatic damages together with inflammatory activity. This might be termed chemotherapy-associated steatohepatitis (CASH), a most severe form of non-alcoholic fatty liver disease. Therefore, in the present study, we aimed to compare the effect of antioxidant compounds on simultaneous development of oxaliplatin-induced hepato and neurotoxicity in mice. **Methods:** The Balb/c mice were treated with doses of oxaliplatin (OXA) for 6 weeks, 10 mg/kg, intraperitoneally (i.p), resulting in mechanical allodynia, and hepatic steatosis. We administered antioxidants compounds such as rutin (RUT) (20 mg/Kg/d), resveratrol (RVS) (100 mg/Kg/d), quercetin (QT) (20 mg/Kg/d) and nanoquercetin (NQT) (20 mg/Kg/d) daily by gavage to Balb/c. *N*-acetyl-cysteine was used as control. Euthanasia occurred on day 43 after treatment. We evaluated mechanical nociceptive threshold, ALT/AST, histopathological analysis and MPO activity. Statistical analyses were made one way ANOVA, followed by Bonferroni post hoc test. **Results:** The treatments with RSV, RUT or NQT were able to prevent mechanical allodynia when compared to OXA group. Regarding the effect on steatohepatitis, resveratrol, quercetin and quercetin nanoemulsion almost completely reversed the mean liver weight increase by OXA. In accordance with these previous data, histological evaluation depicted attenuation all features of hepatic steatosis evaluated in resveratrol, rutin, quercetin and quercetin nanoemulsion groups. On the other hand, only quercetin and quercetin nanoemulsion treatments were able to reduce neutrophils migration measured by MPO activity. **Conclusion:** These results suggest that the use of compounds such as resveratrol, rutin,

quercetin and quercetin nanoemulsion can be effective to avoid oxaliplatin-inducing hepato and neurotoxicity in a rodent model.

**Keywords:** Oxaliplatin, mechanical allodynia, steatohepatitis, antioxidants.



## LISTA DE ABREVIATURAS

**AVC:** acidente vascular cerebral

**CASH:** *chemotherapy-associated steatohepatitis*

**CIPN:** *chemotherapy induced peripheral neuropathy*

**CRC:** câncer colorretal avançado

**FA:** *folic acid*, ácido fólico

**5-FU:** 5-fluorouracil

**FDA:** *Food and Drug Administration*

**FOLFOX:** ácido folínico/fluorouracil/oxaliplatina

**GLU:** glicose

**H<sub>2</sub>O<sub>2</sub>:** peróxido de hidrogênio

**HCO<sub>3</sub><sup>-</sup>:** bicarbonato

**H<sub>2</sub>PO<sub>4</sub>:** dihidrogênio fosfato

**HE:** hematoxilina-eosina

**iNOS:** óxido nítrico sintase induzível

**MPO:** mieloperoxidase

**NAFLD:** *non-alcoholic fatty liver disease*

**NASH:** *non-alcoholic steatohepatitis*

**NQT:** quercetina em nanoemulsão

**OXA:** oxaliplatina

**Pt:** platino

**Pt(dach)Cl<sub>2</sub>:** Dicloro(1,2 –diaminociclohexano) platino

**Pt(dach)Cl:** Monocloro(1,2 –diaminociclohexano) platino

**QT:** quercetina

**ROS:** espécies reativas de oxigênio

**RSV:** resveratrol

**RUT:** rutina

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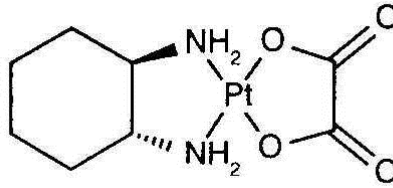
# 1. INTRODUÇÃO

## 1.1 OXALIPLATINA

Diversos fármacos quimioterápicos vêm sendo utilizadas para o tratamento de câncer, especialmente no câncer colorretal. Entre os fármacos mais utilizados, encontra-se a oxaliplatina, que é um agente quimioterápico alquilante de terceira geração capaz de inibir a síntese e replicação de DNA, formando adutos com o mesmo (Cardus *et al.*, 2009).

No momento em que a oxaliplatina entra na corrente sanguínea o  $\text{HCNO}_3^-$  e  $\text{H}_2\text{PO}_4^-$  desacoplam seu grupamento oxalato, formando intermediários não - estáveis, que rapidamente se hidrolisam a espécies platinas como dicloro (1,2-dach) platino  $\text{Pt}(\text{dach})\text{Cl}_2$  e monoclora (1,2-dach) platino  $\text{Pt}(\text{dach})\text{Cl}$ , que instantaneamente reagem com o DNA celular, proteínas e outras macromoléculas levando a apoptose celular (Foltinová *et al.*, 2008). O grupo alquilante pode formar ligações cruzadas entre dois sítios nucleofílicos, como o nitrogênio na posição (N-7) da guanina no DNA, originando ligações cruzadas intrafilamentares e interfilamentares, Foltinová *et al.*, 2008). Seu principal efeito é observado durante a síntese de DNA (Rang *et al.*, 2004). A citotoxicidade é inespecífica ao ciclo celular (Goldman *et al.*, 2009).

A oxaliplatina (*cis*- [(1*R*,2*R*)-1,2-cyclohexanediamine-*N,N'*] oxalato (2-)-*O,O'*] platinum) é uma substância com peso molecular 397.3 e sua fórmula molecular ( $\text{C}_8\text{H}_{14}\text{N}_2\text{O}_4\text{Pt}$ ) (Figura 1) confere a sua baixa solubilidade em água, menor solubilidade em metanol e ainda, sua quase total insolubilidade em etanol e acetona (Alcindor *et al.*, 2011).



**Figura 1.** Estrutura molecular da oxaliplatina.

Fonte: Graham *et al.*, 2000.

A oxaliplatina tem sido utilizada como terapia para o tratamento de alguns tipos de câncer, devido a uma ampla atividade antitumoral. Ela tem se mostrado ativa no câncer colorretal, no câncer de ovário, no câncer de células germinativas e no câncer cervical (Goodman & Gilman's, 2001; Renn *et al.*, 2011). Quando usada para tratar câncer colorretal avançado (CRC), ela é combinada com 5-fluorouracil (5-FU), uma fluoropirimidina, e com leucovorina (LV), num regime conhecido como FOLFOX (Suzuki *et al.*, 2013; Baekk *et al.*, 2010).

O tratamento quimioterápico pré-operatório com a oxaliplatina é cada vez mais utilizado em pacientes com metástases hepáticas, cujo sítio primário se localiza na porção colorretal (Choti *et al.*, 2009) e para o tratamento de pacientes submetidos à hepatectomia extensa, particularmente com tumores grandes ou tumores em localização desfavorável. No entanto, essa terapia pode contribuir para o desenvolvimento de uma forma de esteato-hepatite (CASH- *chemotherapy – associated steatohepatitis*), além de estar relacionada com a ocorrência de uma dilatação sinusoidal, com o aumento do risco de

infecções e de mortalidade após a ressecção de metástases hepáticas (Hebbar *et al.*, 2009; Morris-Stiff *et al.*, 2007).

A lesão sinusoidal é caracterizada por dilatação sinusoidal, congestão de eritrócitos e, ocasionalmente, é acompanhada por fibrose e oclusão venular fibrótica perisinusoidal (Choti, 2009). Essa lesão parece ter uma clara associação com o uso de oxaliplatina (Choti 2009; Vauthey *et al.*, 2006).

Por outro lado, a esteatose hepática induzida por agentes quimioterápicos leva a dano hepático significativo e lesões vasculares (Hebbar *et al.*, 2009), os quais aumentam o risco de morbidade peri-operatória e a taxa de mortalidade (Keizman *et al.*, 2010). Essa esteato-hepatite é caracterizada pelo acúmulo de lipídeos/gordura no fígado, o que pode ser considerado patogênico quando o conteúdo hepático de gordura for superior a 5 % do peso do fígado (Kahn *et al.*, 2009).

## **1.2 ESTEATOSE HEPÁTICA E NEUROTOXICIDADE**

Fernandez e colaboradores (2005) verificaram que em biópsias de pacientes tratados com oxaliplatina, foi constatada hepatite moderada ou grave, aumentando as evidências acerca dos efeitos da oxaliplatina sobre a esteatose hepática (Fernandez *et al.*, 2005). A esteatose hepática não alcoólica, (NASH – *non-alcoholic steatohepatitis*) associada à quimioterapia pode ser resultante da deposição de gordura no fígado resultante de outras causas que não o consumo de álcool (NAFLD – *non-alcoholic fatty liver disease*) conforme Kelishadi, 2013. A NASH é a forma mais grave de NAFLD, uma vez que a infiltração gordurosa no fígado é acompanhada por necrose e

inflamação (Kanuri *et al.*,2013) podendo levar a uma diminuição significativa da função hepática (Cleary *et al.*, 2009).

A ocorrência de NASH é clinicamente importante, pois 15 a 25 % dos pacientes têm uma evolução para cirrose. Uma vez que a cirrose é estabelecida, estima-se que 30 a 40 % dos pacientes virão a óbito ao longo de um período de 10 anos (Shifflet *et al.*, 2009).

As características histológicas da esteato-hepatite incluem, em diferentes graus, esteatose/deposição de gordura, inflamação lobular e balonização dos hepatócitos (Kahn *et al.*, 2009; Tarantino *et al.*, 2013). Quando este quadro é associado com a realização de quimioterapia para o câncer, o mesmo é denominado de CASH (Kahn *et al.*, 2009).

Os agentes derivados da platina, incluindo cisplatina, carboplatina e oxaliplatina, também são conhecidos por causar sintomas clássicos de neuropatia periférica induzida por quimioterapia (CIPN – *chemotherapy induced peripheral neuropathy*). Embora a CIPN esteja associada aos três derivados da platina, a maior incidência é observada com o uso da cisplatina e da oxaliplatina (Ogawa *et al.*, 2013).

A neurotoxicidade decorrente da terapia com a oxaliplatina pode ser dividida em duas síndromes distintas. A primeira é de fase aguda, devido à hiperexcitabilidade transitória dos nervos periféricos, ocorrendo logo após a infusão de oxaliplatina. Entre os derivados da platina, este é um efeito único da oxaliplatina (Wang *et al.*, 2007). Esta forma de neuropatia ocorre normalmente com baixas doses cumulativas totais, pode ser desencadeada ou agravada pela exposição ao frio, é sempre reversível e, geralmente, não requer a descontinuação da terapia (Aoki *et al.*, 2012). Os pacientes podem apresentar

parestesia e disestesia das mãos e dos pés, bem como, da laringe e da mandíbula (Schellingerhout *et al.*, 2012). Estes sintomas geralmente ocorrem depois de horas da exposição e são reversíveis após algumas horas ou nos dias seguintes à administração da oxaliplatina (Sprowl, *et al.*, 2013). Segundo Sittl e colaboradores (2012), após algumas horas ou dias da infusão de oxaliplatina, cerca de 80% dos pacientes apresentarão parestesias distais e contrações musculares leves. Manifestações menos comuns incluem alterações visuais, alterações na voz e parestesia perioral (Kannarkat *et al.*, 2007). Presumivelmente, o oxalato, metabólito da oxaliplatina, quelata o cálcio e magnésio, afeta os canais de sódio neuronais voltagem-dependentes, causando os efeitos mencionados acima (Kawashiri *et al.*, 2012).

No entanto, a segunda síndrome, conhecida como neuropatia sensorial periférica, possui sintomas semelhantes aos observados com o uso da cisplatina, ocorrendo principalmente nas extremidades distais. Esta forma de neurotoxicidade possui implicações clínicas mais importantes, e podem durar vários meses, resultando em uma perturbação grave da função (Wang *et al.*, 2007). A neuropatia sensorial periférica é induzida pela administração de múltiplas doses de oxaliplatina (Jamieson *et al.*, 2005) e está relacionada com o efeito cumulativo destas doses, tornando-se comumente um problema clínico, quando a dose cumulativa se aproxima de 800 mg/m<sup>2</sup> (Amptoulach *et al.*, 2011).

Um dos mecanismos sugeridos para a neurotoxicidade induzida pelos derivados da platina é devido ao seu acúmulo no sistema nervoso periférico, principalmente na raiz dorsal dos gânglios, gerando espécies reativas de oxigênio (Renn *et al.*, 2011). A presença patológica de oxaliplatina no sistema

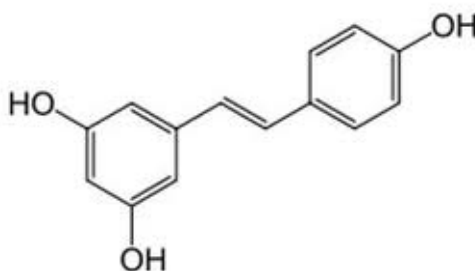


nervoso periférico ocorre devido a eliminação lenta da droga (Holmes *et al.*, 1998). Park e colaboradores (2009) mostraram que as espécies reativas de oxigênio geradas pelos compostos da platina desempenham um importante papel na morte celular por apoptose das células neuronais via sinalização da p53 (Park *et al.*, 2009).

O FDA (*Food and Drug Administration*) observou que mais de 70% dos pacientes que fizeram uso de oxaliplatina são afetados por algum grau de neuropatia sensorial, sendo muitas vezes causa da interrupção do tratamento (McWhinney *et al.*, 2009).

### 1.3 AGENTES ANTIOXIDANTES RESVERATROL E RUTINA

O resveratrol (trans- 3, 5, 4' trihidroxiestilbeno) é um composto polifenólico natural, de estrutura molecular relativamente simples que interage com diversos alvos moleculares (Figura 3) (Leiro *et al.*, 2010; Svajger *et al.*, 2012). Ele é encontrado em várias fontes alimentares, tais como uvas, frutas, amendoim, bem como no vinho tinto (Souvik *et al.*, 2011).



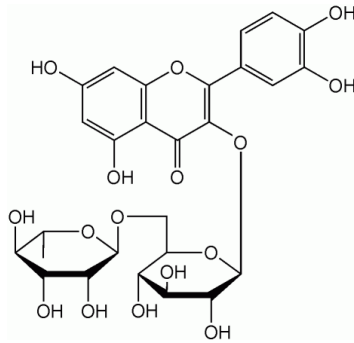
**Figura 2.** Estrutura molecular do resveratrol.

Fonte: Dan Su *et al.*, 2013

Na natureza, o resveratrol funciona como um fungicida produzido pela própria planta para repelir organismos potencialmente letais e combater o estresse ambiental. O seu valor benéfico para a saúde humana está bem documentado (Bishayee *et al.*, 2010). Estudos mostram que ele apresenta uma grande variedade de ações farmacológicas, incluindo efeitos anti-inflamatórios, podendo prevenir ou retardar a progressão de diversas doenças relacionadas com a inflamação, incluindo câncer, diabetes, doenças neurodegenerativas, doenças cardiovasculares, lesões isquêmicas, infecções virais, bem como, aumentar a resistência ao estresse, modulando, ainda, a atividade de enzimas antioxidantes (Venturini *et al.*, 2010; Bishayee *et al.*, 2010).

Achados experimentais revelam múltiplos alvos celulares para o resveratrol que podem afetar o crescimento e proliferação celular, apoptose, inflamação, invasão, angiogênese e metástase (Ganaphaty *et al.*, 2010). Nas células de câncer colorretal, o resveratrol apresentou atividade inibitória de crescimento, diferenciação e proliferação celular e induziu a apoptose (Araújo *et al.*, 2011). Atualmente, encontra-se resveratrol disponível no mercado na forma de comprimidos como suplemento dietético seguro e bem tolerado (Bhatt *et al.*, 2012, Fraczeck *et al.*, 2012).

A rutina é uma glicosil flavona (Figura 4) presente em abundância em plantas como em sementes de trigo sarraceno, frutas e cascas de frutas, especialmente frutas cítricas (Isai *et al.*, 2009).



**Figura 3.** Estrutura molecular da rutina.

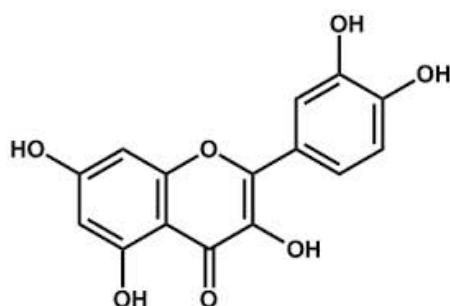
Fonte: Rahmat *et al.*, 2012

A rutina demonstrou apresentar atividade antioxidante e diminuir os níveis de colesterol hepático e sanguíneo (Sun *et al.*, 2011). Além de apresentar atividade antiplaquetária. A rutina também modula uma série de funções biológicas e apresenta atividades anti-inflamatória, antialérgica, antimicrobiana e propriedades vasoativas (Almeida *et al.*, 2010). A capacidade da rutina em eliminar os radicais e inibir a peroxidação lipídica tem sido relatada em condições de estresse oxidativo induzido por estreptozotocina, um agente indutor de diabetes do tipo I (Isai *et al.*, 2009). A rutina suprime processos mediados por radicais (Rahmat *et al.*, 2012). Além disso, esta substância também parece desempenhar um papel positivo no metabolismo de carboidratos, por aumentar a capacidade antioxidante de ratos diabéticos (Isai *et al.*, 2009). Estudo histopatológico revelou mínimo dano tecidual e menor número de depósitos de oxalato de cálcio nos rins dos animais tratados com rutina e curcumina em relação aos animais não tratados. Os dados sugerem que a rutina e curcumina inibem a urolitíase de oxalato de cálcio (Ghudasara *et al.*, 2010). O estudo de Boeira *et al.*, 2011 revelou os efeitos benéficos dos compostos rutina e quercetina na prevenção de alterações inflamatórias e

nociceptivas causadas pela cistite hemorrágica em camundongos que utilizaram ciclofosfamida quando comparados com os grupos tratados com Mesna.

#### 1.4 QUERCETINA E QUERCETINA EM NANOEMULSÃO

A quercetina (3,5,7,3'-4'-pentahidroxi flavona) é um flavonóide que se encontra presente na dieta humana. Os flavonóides são substâncias fenólicas naturais amplamente presentes em legumes, frutas, óleo de oliva, própolis de abelha e bebidas como o chá e o vinho tinto, podendo atuar como agentes antioxidantes em sistemas biológicos. (Sestili *et al.*, 1998; Mi *et al.*, 2010). Além da dieta, a quercetina pode também ser encontrada no *Ginkgo biloba*, *Hypericum perforatum* e *Sambucus canadensis* (USDA, 2011) e possui uma variedade de atividades biológicas (Mi *et al.*, 2010), além de funcionar como um quelante de metais, incluindo o ferro (Sestili *et al.*, 1998; Mi *et al.*, 2010).



**Figura 4.** Estrutura molecular da quercetina

Fonte: Pecivová *et al.*, 2012

Vários estudos têm indicado que a quercetina inibe a expressão das enzimas ciclooxigenase induzível (COX-2) e óxido nítrico sintase induzível (iNOS), inibe também a xantina oxidase, a peroxidação lipídica e alguns danos

ao DNA causados por H<sub>2</sub>O<sub>2</sub> (Mi *et al.*, 2010). Adicionalmente, possui efeitos antinociceptivos (Valério *et al.*, 2009). Ensaio *in vitro* e *in vivo*, tem demonstrado que a quercetina e seus derivados, como outros flavonóides, possuem ação antioxidante, antimutagênica e antitumoral (Atawodi *et al.*, 2009), diminuindo a citotoxicidade causada pela administração de doxorubicina a células de fígados normais (Wang *et al.*, 2012). Este composto tem atraído considerável atenção por parecer promissor para a terapia e prevenção de doenças cardíacas e também a diminuição da incidência de AVC (Pashevin *et al.*, 2011). Além disso, demonstrou inibir o crescimento e a proliferação de células de câncer colo retal (HT-29, COLO 201, LS 174T, HCT-116, SW 480 e Caco-2) induzindo apoptose nestes tipos celulares (Araújo *et al.*, 2011).

Apesar de suas atividades benéficas, a quercetina possui baixa solubilidade em água que resulta na fraca absorção, baixa biodisponibilidade e, portanto, limitando seu potencial na aplicação clínica (Wang *et al.*, 2012).

Visto que as características químicas inerentes a quercetina podem limitar seus efeitos no organismo, novas preparações procuram melhorar a solubilidade da quercetina através da adição de emulsões lipossomais aproveitando suas propriedades terapêuticas, pois permite o controle da liberação do fármaco e a capacidade de proteger o princípio ativo da degradação (Dora *et al.*, 2011).

O Instituto Nacional do Câncer reconheceu a nanotecnologia como um campo emergente para revolucionar a detecção, prevenção e tratamento do câncer (Kawasaki, *et al.*, 2005).

As nanopartículas apresentam alta capacidade de transporte e de viabilidade de várias vias de administração, inclusive oral, proporcionando a liberação do princípio ativo de forma sustentável no organismo (Aparajita *et al.*, 2013).

A capacidade que o sistema lipídico tem de incorporar a quercetina foi avaliada por um método analítico de espectroscopia UV que foi desenvolvido e validado por Dora e colaboradores (2011). Na etapa de validação da técnica foram avaliados os parâmetros de linearidade, limites de detecção e quantificação e especificidade. As análises da amostra branca demonstraram que o método foi específico, e não houve nenhuma interferência ou sobreposição dos excipientes com as absorvâncias de nanoemulsão contendo quercetina (Dora *et al.*, 2011). A nanoemulsão contendo quercetina desenvolvida apresentou um teor de fármaco de aproximadamente 1000 µg/mL e uma recuperação de 98 %, indicando uma excelente capacidade de solubilizar o fármaco devido sua grande área interfacial, sendo que houve um aumento de solubilidade de 2720 vezes se comparado com a solubilidade da quercetina em água (Dora *et al.*, 2011). Sabe-se que o tamanho e a distribuição das nanopartículas são determinantes para sua estabilidade e eficácia na absorção celular (Aparajita *et al.*, 2013).

## **2 OBJETIVOS**

### **2.1 OBJETIVO GERAL**

O presente estudo teve como objetivo testar o efeito do resveratrol, rutina, quercetina e quercetina em nanoemulsão sobre a neuro e hepatotoxicidade induzidas pela oxaliplatina em camundongos.

### **2.2 OBJETIVOS ESPECÍFICOS**

- a. Padronizar o modelo de esteatose hepática e de neurotoxicidade induzida pelo quimioterápico oxaliplatina em camundongos, a fim de avaliar os efeitos de novas estratégias com potencial hepato e neuroprotetor;
- b. Avaliar os efeitos dos compostos resveratrol, rutina, quercetina e quercetina em nanoemulsão sobre a alodínia através da aplicação do monofilamento de Von Frey;
- c. Verificar os efeitos dos compostos resveratrol, rutina, quercetina e quercetina em nanoemulsão sobre a esteatose hepática induzida pela oxaliplatina, através de avaliação macroscópica e histopatológica;
- d. Analisar os efeitos dos compostos resveratrol, rutina, quercetina e quercetina em nanoemulsão sobre a atividade das enzimas séricas AST e ALT;
- e. Determinar os efeitos dos compostos resveratrol, rutina, quercetina e quercetina em nanoemulsão sobre a migração de neutrófilos pela medida da atividade da mieloperoxidase (MPO), em fígados de camundongos tratados com oxaliplatina.

### 3. ARTIGO CIENTÍFICO

Os resultados do presente trabalho foram submetidos ao periódico  
Naunyn-Schmiedeberg's Archives of Pharmacology  
Fator de Impacto (ISI Web of Knowledge): 2.10



**Effects of the compounds resveratrol, rutin, quercetin and quercetin nanoemulsion on oxaliplatin-induced hepatotoxicity and neurotoxicity in mice**

**Running Head: quercetin, resveratrol and rutin effects in neurotoxicity and steatohepatitis**

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## ABSTRACT

Oxaliplatin (OXA) is a third-generation platinum compound developed with the purpose of overcoming the limitations of toxicity, tumor resistance and poor oral bioavailability associated to cisplatin administration. Despite its usefulness, oxaliplatin-associated neurotoxicity represents the main dose limiting factor of this drug and until now there is not suitable treatment. Chemotherapy with oxaliplatin also increases the rate of developing hepatic damages with inflammatory activity, termed chemotherapy-associated steatohepatitis (CASH). In the present study, we aimed to compare the effect of a series of antioxidant compounds on simultaneous development of oxaliplatin-induced hepato and neurotoxicity in mice. Mice Balb/c were treated with oxaliplatin for 6 weeks, 10 mg/kg, intraperitoneally, resulting in mechanical allodynia and hepatic steatosis. We administered antioxidant compounds such as rutin (RT) (20 mg/Kg), resveratrol (RVS) (100 mg/Kg), quercetin (QT) (20 mg/Kg) and quercetin nanoemulsion (NQT) (20 mg/Kg) daily by gavage to Balb/c, and *N*-acetylcysteine (NAC) was used as positive control. The treatments with RSV, RUT or NQT were able to prevent mechanical allodynia when compared to OXA group. Regarding the effect on steatohepatitis, RVS, QT and NQT almost completely reversed the mean liver weight increase by OXA. In accordance with these previous data, histological evaluation depicted attenuation all the features of hepatic steatosis evaluated in RSV, RUT, QT and NQT groups. On the other hand, only QT and NQT treatments were able to reduce neutrophils migration measured by MPO activity. These results suggest that the compounds tested

RSV, RU, QT and NQT would be useful for the clinical treatment of neuro and hepatotoxicity induced by oxaliplatin.

**Keywords:** CASH, neuropathy, polyphenol compounds, oxaliplatin

## Introduction

Oxaliplatin is a third-generation platinum compound developed with the purpose of overcoming the limitations of toxicity, tumor resistance and poor oral bioavailability associated to cisplatin administration (Shah and Dizon, 2009). Oxaliplatin is used in combination with other chemotherapy agents, such as 5-fluorouracil/folinic acid, leucovorin, irinotecan, and represents a first-line therapy on colorectal cancer (Culy et al., 2000). This regimen therapy also includes management options of colorectal liver metastasis (Brouquet et al., 2009), ovarian (Lee et al., 2013), gastric (Kim et al., 2012), and non-small cell lung cancer (Shi et al., 2013). Despite its usefulness, this platinum compound causes severe side effects. Oxaliplatin-associated neurotoxicity represents the main dose limiting of this drug and, until now, there is no suitable treatment (Park et al., 2009). Oxaliplatin might be involved in vascular sinusoidal injury and neurotoxicity, with the development of peripheral sensory neuropathy, which symptoms are characterized by dysesthesia and paraesthesia of extremities and increase with higher doses of oxaliplatin (Ali, 2010; Amptoulach and Tsavaris, 2011; Culy et al., 2000).

Oxaliplatin also augments the rate of developing hepatic damage with increased inflammatory activity (Choti, 2009). This might be termed chemotherapy-associated steatohepatitis (CASH), a most severe form of non-alcoholic fatty liver disease (NAFLD) (Malaguarnera et al., 2009). The liver injury in steatohepatitis presents fatty liver infiltration together with liver necrosis, and it is characterized by the presence of cellular ballooning, micro and macrovesicular steatosis and acinar and lobular inflammation (Zorzi et al., 2007). Another pathogenic factor related to steatohepatitis is the overproduction

of free radicals, the reactive oxygen species (ROS), owing to oxidative stress that contribute to further hepatic injury (Sakaguchi et al., 2011). Different ROS types are able to cause oxidative damage to the DNA, protein and fatty acids (Koek et al., 2011). Events like lipid peroxidation and reduction of anti-oxidant enzymes enhance the oxidative stress including platinum-induced toxicity (Carozzi et al., 2010). Conversely, there are some antioxidative defenses, enzymatic and non-enzymatic, in order to avoid or reverse this damage by means of scavenging the ROS (Apel and Hirt, 2004; Rezazadeh et al., 2012). On the other side, several antioxidants compounds, from diet or produced endogenously, had shown amelioration of the steatohepatitis and oxaliplatin-induced neurotoxicity via reducing the oxidative stress (Koek et al., 2011).

Flavonoids are natural polyphenol substances reported for their powerful protective activities on different tissues and for their activity against cancer progression, especially because their antioxidant activities or scavenging properties. They are present in vegetables, fruits, olive oil, bee propolis and beverages such as green tea and red wine (Mi et al., 2010; Sestili et al., 1998). Quercetin, resveratrol and rutin are the major dietary polyphenol compounds with potent antioxidant activities. Resveratrol can interact with multiple molecular targets due its simply molecular structure (Calamini et al., 2010; Delmas et al., 2013; Leiro et al., 2010; Svajger and Jeras, 2012). Recent findings reveal neoplastic cell growth and proliferation, apoptosis (Ikeda et al., 2013), inflammation (Leiro et al., 2010), invasion (Chen et al., 2013), angiogenesis and metastasis (Ganapathy et al., 2010) as cellular targets of resveratrol. Presently, resveratrol is commercially available as a safe and well tolerated dietary supplement (Bhatt et al., 2012). Rutin is a glycoside flavone

that also modulates a variety of biological functions having activities in oxidative stress (Patil et al., 2013), inflammatory process and in the reduction of blood and hepatic cholesterol (Al-Rejaie et al., 2013). Its ability in scavenging free radicals and inhibiting lipid peroxidation implicated in the etiology of some pathologies has been shown (Patil et al., 2013). Quercetin has a variety of actions besides the antioxidant activity, highlighted by antimutagenic (Gupta et al., 2010) and antitumorigenic (Wang et al., 2012b) properties. Despite its benefits, quercetin has low solubility in water resulting in poor absorption, low bioavailability and therefore limiting its potential clinical application (Aebi, 1984; Wang et al., 2013; Wang et al., 2012a). Thus, the development of a drug delivery system containing quercetin can improve its solubility, absorption, and therapeutic index (Dora et al., 2012).

Currently there are few pharmacological options available of steatohepatitis and peripheral sensory neuropathy induced by oxaliplatin, making necessary to identify new nontoxic and effective pharmacological alternatives for the treatment of these disorders. Therefore, the aim of the study was to compare the effect of the compounds resveratrol, rutin, quercetin and quercetin nanoemulsion on simultaneous development of oxaliplatin-induced hepato and neurotoxicity in mice.

## **Materials and Methods**

### ***Drugs and reagents***

The following drugs and reagents were used: oxaliplatin (Sanofi-Aventis, France); carboxymethylcellulose (Natural Pharma), hydrogenated soybean lecithin (Lipoid), 12-hydroxystearic acid–polyethylene glycol copolymer (BASF),

hexadecyltrimethyl ammonium bromide, *N*-acetylcysteine (NAC), castor oil and tetramethylbenzidine (Sigma-Aldrich, St. Louis, USA). NaPO<sub>4</sub>, H<sub>2</sub>O<sub>2</sub>, NaCl and Tween 20 were all purchased from Merck (Haar, Germany). The compounds resveratrol (RSV), rutin (RU) and quercetin (QT) were obtained from Sigma Chemical Co. (purity grade >95%). Quercetin nanoemulsion (NQT) was synthesized in the Laboratory for Nanotechnology Applied to Health, Federal University of Rio Grande, Brazil. The compounds dilutions were made in NaCl 0.9% (saline solution). Quercetin was diluted in carboxymethylcellulose 0,2%.

### ***Preparation of nano quercetin by solvent diffusion technique hot***

The quercetin nanoemulsion (NQT) was prepared by a hot solvent diffusion method associated to phase inversion technique (Dora et al., 2012). Briefly, a solution containing quercetin, castor oil and lecithin in acetone: ethanol (60:40, v / v) at 60°C was added an aqueous phase containing surfactant 12-hydroxystearic acid–polyethylene glycol copolymer previously heated at 80°C under magnetic stirring at 700 rpm. The resulting colloidal dispersion was cooled to room temperature under magnetic stirring, the solvent was evaporated under reduced pressure, and the final volume was adjusted to 20 mL. Finally, the dispersion was filtered with an 8 microns membrane. The formulations were made in triplicate. The quercetin nanoemulsion characterizations were (i) analysis of particle size and polydispersity index (PDI) by dynamic light scattering (Zetasizer), (ii) analysis of zeta potential by Laser-Doppler anemometry (Zetaziser) and (iii) analysis of quercetin content and recovery by UV spectrophotometric method.

## **Animals**

Male BALB/c mice (25 to 30 g) were obtained from the Federal University of Pelotas (Brazil). All animals were maintained under controlled temperature ( $22\pm 2^{\circ}\text{C}$ ) and humidity (60 to 70%), with 12-hour light-dark cycle, with food and water *ad libitum*. All animal procedures were performed according with the Guide for the Care and use of Laboratory Animals, published by National Institutes for Health (NIH). The experimental protocol was approved by the Local Animal Ethics Committee (approval number 10/00168). The number of animals and the intensity of noxious stimuli were the minimum necessary to demonstrate the consistent effects of the drug treatments.

## ***Oxaliplatin-induced hepato- and neurotoxicity model in mice***

The model to induce steatohepatitis (SH) and neurotoxicity in mice was adapted from Keizman et al (2010). For the OXA group, mice received six injections of oxaliplatin (10 mg/kg, i.p.), or saline (10 ml/kg, i.p.) for the control group (SAL), on days 1, 8, 15, 22, 29 and 36. On day 43, the animals were euthanized by deep anesthesia with isoflurane and the samples (blood and liver) were collected for further analysis.

## ***Experimental design***

The animals were randomly divided into eight treatment groups (n=5): i) SAL+SAL; ii) OXA+ SAL; iii) OXA+ NAC (200 mg/kg, p.o.) (Thong-Ngam et al., 2007); iv) OXA+ RSV (100 mg/kg, po); v) OXA+RU (20 mg/kg, po); vi) OXA + WN (10 ml/kg, p.o.), vii) OXA+ QT (20 mg/kg, p.o.); and viii) OXA+ NQT (20 mg/kg, p.o.). All compounds were administrated daily, during 42 days.



Behavioral tests (mechanical allodynia) were performed weekly, after 30 min post oxaliplatin injection. The animals were fed daily until the day 42 and were weighed weekly.

### ***Mechanical nociceptive threshold***

To evaluate mechanical allodynia threshold, Von Frey monofilaments (Stoelting, USA) corresponding to 0.6 g were applied up vertically into the right hind paw of mice with sufficient pressure. The mice were housed 30 min before the measurement on a wire mesh floor under an acrylic box. Some criteria were followed: i) paw withdrawal was considered when the animal completely remove its paw, ii) each animal was stimulated for 10 times with duration of 5 s in each stimulation, iii) each withdrawal was recorded as 10% of response.

### ***Histopathological analysis***

For histological tissue analysis, the tissues removed were fixed in 10% formalin, processed and then were dehydrated in increasing concentrations of ethanol (70-100%) and embedded in paraffin. Sections of 5  $\mu$ m were made and the slides were stained and mounted in Entellan (Merck, SP, Brazil) at the Laboratory of Pathology of UFCSPA. Livers were submitted to hematoxilin and eosin (H&E) staining, performed according standard protocol. The samples were examined by a pathologist blinded to the experimental groups on a fluorescent microscope (Zeiss Axioskop 40 equipped with a CoolSnap Pro of CCD camera (Media Cybernetics, Rockville, USA) based on criteria described by Tannapfel et al ( 2010). All images were obtained under magnification of 400X and in a single day, with white, black level calibration and optical density

calibration performed and recorded to avoid any shifts in the calibration of light parameters of optical microscope and thus, maintaining the same configurations for all images. A total of ten images were used for quantitative assessment in the Image Pro-Plus 4.5.1 (Media Cybernetics, Rockville, USA). Density was evaluated as integrated optical density (IOD) (area x optical density), and roundness was counted as  $\text{perimeter}^2/4 \times \text{PI} \times \text{area}$ .

### ***Myeloperoxidase activity***

Myeloperoxidase (MPO) was measured according to the method described by (Fernandes et al., 2003), to verify neutrophil recruitment. Portion of the liver was frozen at  $-80^{\circ}\text{C}$  immediately after removed. Before analyses the tissues were partially thawed and homogenized into an ice-containing box in 5% EDTA buffer/NaCl solution (pH 4.7) and centrifuged at 10.000 RPM for 15 minutes,  $4^{\circ}\text{C}$ . The pellet was suspended in 0.5% hexadecylmetil ammonium bromide buffer (pH 5.4) and the samples were centrifuged (10.000 RPM, 15 min,  $4^{\circ}\text{C}$ ). The supernatant was used (20  $\mu\text{l}$ ) for enzymatic reaction of MPO. Detection was carried out in the presence of tetramethylbenzidine (1.6 mM),  $\text{Na}_3\text{PO}_4$  (80 mM) and hydrogen peroxide (0.3 mM). The absorbance was measured at 595 nm and results expressed as optical density (OD) per mg of tissue.

### ***Serum analysis of AST and ALT***

Whole blood was centrifuged at 300 g for 10 min; the supernatant corresponding to the serum was removed and frozen at  $-80^{\circ}\text{C}$  until analysis. The assays to measure alanine-aminotransferase (ALT) and aspartate-

aminotransferase (AST), as indirect indicators of liver damage, were performed following the requirements of manufacturer (Labtest, Brazil). The results were expressed in U/L.

### **Statistical analysis**

Data are presented as mean  $\pm$  SEM. Statistical analysis were made by one-way analysis of variance (ANOVA), followed by Bonferroni *post hoc* test. P values < 0.05 were considered as significant. Statistical calculations were performed using GraphPad software (GraphPad InStat, version 4, USA).

### **Results**

In this study we have evaluated the effect of different antioxidant compounds in neurotoxicity induced by oxaliplatin injection in mice. The baseline withdrawal thresholds in the mechanical tests in all experimental groups before OXA injection were similar. Oxaliplatin (10 mg/kg, i.p., once a week for 6 weeks) significantly increased the frequency of paw withdrawal compared to the control group in the von Frey test on day 42 ( $p < 0.0001$ ). This significant increased frequency had its onset on the 4<sup>th</sup> week and persisted throughout the weeks 5 and 6 of OXA-treatment (Figure 1A). Interestingly, all antioxidants treated groups, reverted the increase of the paw withdrawal responses caused by OXA injection on day 42, in a similar way of the positive control NAC (Figure 1B). WN and QT groups did not significantly affect the mechanical allodynia in any evaluated period of time.

In order to evaluate hepatotoxicity in this mice model induced by OXA, body and liver weights were analyzed. Mean variation of body weight gain of

animals increased over time in control (SAL) and QT-treated group, but remained unchanged in all other groups (Figure 2A). No animal died during the course of the experiments. Mean liver weight in OXA group increased 20.3% relative to control group, and when mice were treated with NAC, RSV, QT and NQT liver weight reduced significantly (11%, 21% e 17.5%, respectively) in comparison to OXA group (Figure 2B).

Histological examination of the liver sample slides stained with H&E was made by quantitative and qualitative analysis. Histopathological findings were featured according to the following changes: steatosis, hepatocyte ballooning and inflammation. Ballooned hepatocytes were seen mainly in OXA and WN group (Figure 3B and 3F). Their microscopic features are presented as enlarged hepatocytes with swollen vacuolated cytoplasm and the hyperchromatic nucleus occasionally appears with a prominent nucleolus. Sinusoidal dilatation between the hepatocytes in these groups was also observed. In addition, an attenuation of all the features above mentioned in NAC, RSV, RUT, QT and NQT groups (Figures 3C, D, E, G and H, respectively) was detected. Performing quantitative analysis, the parameters used to compare the differences among the groups were: area, density, roundness. The cytoplasmatic area and total optical density area analysis showed a significant reduction of the area in OXA group when compared to SAL group; on the other hand the roundness parameter was not different among the groups. All treated groups augmented area and density when compared to OXA group (Table 1).

OXA treatment increased significantly MPO activity related to the control group ( $p < 0.01$ ). Interestingly, QT and NQT treatments were able to reduce

neutrophils migration, as indicated by MPO activity ( $p < 0.05$ , Figure 4). We did not observe any differences in the other groups.

Assessment of transaminases levels (AST and ALT) did not reveal any significant alteration in mice blood serum after the treatments, although, there was a clear tendency of diminishing ALT in the treatment groups (Figure 5B).

## **Discussion**

Several studies report that chemotherapy treatment with oxaliplatin can induce the development of liver damage followed by a progressive stage of SH, associated to neurotoxicity and its painful consequences (Culy et al., 2000; Fernandez et al., 2005; Janes et al., 2013; Rubbia-Brandt et al., 2004). Achieving chemotherapy side effects resolution is a priority and, therefore, the present study assessed the effects of the antioxidant compounds resveratrol, rutin, quercetin and quercetin nanoemulsion on steatohepatitis and neurotoxicity induced by OXA in mice. The mice model presented herein has shown to be suitable of mimicking clinical features induced by oxaliplatin. In fact, in gross evaluation, body and liver sizes of OXA group appeared enlarged, and it was sufficient to produce significant difference in relation to control group (Fig.3).

As mentioned before, neurotoxicity is a very common side effect which has been associated to the use of antineoplastic agents, especially platinum derivatives (Wang et al., 2007). It has been reported that, since the first oxaliplatin administration, neurotoxic symptoms appear yielding acute neuropathy and the ongoing treatment causes cumulative symptoms and worsens the onset neuropathy, featuring chronic neuropathy (Ali, 2010). After long term oxaliplatin administration, the worsening of the symptoms is assigned by loss of sensory

and motor function. These symptoms are usually associated with pain followed by a functional impairment in patients (Deuis et al., 2013). Our oxaliplatin-induced neurotoxicity model resulted in mechanical nociception and the animals treated with OXA once a week presented a higher frequency of paw withdrawal in response to mechanical stimulus through Von Frey filaments application. Of great relevance our data showed that animals receiving OXA plus RSV, RUT, NQT were able to prevent mechanical allodynia when compared to OXA group.

Two main metabolites of oxaliplatin were identified as responsible by these side effects: oxalate and platinum (Sakurai et al., 2009). Oxalate is involved in chelation of extracellular calcium producing an increase in sodium concentration on neurons input in initial stage of neuropathy (Sakurai et al., 2009). Platinum is associated with mechanical allodynia due to its neurotoxicity, causing the formation of morphological changes Pt-DNA in chronic neuropathy, such as lesions of cell bodies, changes in the nucleus and nucleolus, atrophy in neurons of the dorsal root ganglia and cell death (Sakurai et al., 2009). Corroborating to our findings, a previous study from our laboratory has shown that rutin and quercetin produced a significant attenuation of the nociception evoked by the antineoplastic cyclophosphamide in mice (Boeira et al., 2011). Furthermore, quercetin was able to inhibit nociception in a rodent pain model of colitis (Comalada et al., 2005). Outstandingly, resveratrol exhibited an antinociceptive effect related to the prevention of COX-2 mRNA increase in carrageenan-evoked hyperalgesia in rats (Pham-Marcou et al., 2008). In our set of results it is possible to establish a relationship between the mechanical nociception test and pathological examination on regard to the amelioration observed after the treatment in mice, with exception to QT group, which did not

show difference in relation to OXA group indicating that the low QT bioavailability can be a determinant for its use in this condition. On the other hand, quercetin nanoemulsion formulation lowered the allodynia and liver histological parameters presented in the OXA group, demonstrating that this new formulation can be a useful alternative to treat toxicity induced by oxaliplatin. Dora et al. (2011) showed that quercetin nanoemulsion allows a controlled releasing of the drug by reducing undesirable effects, and also increases the drug bioavailability, reaching better therapeutic effects.

We have also investigated the effects of the antioxidant compounds in hepatotoxicity. Antineoplastic drugs such as platinum compounds are involved in the increase of membrane-bounded vacuoles containing degenerative mitochondria, named autophagosome, in nervous tissue; it is believed that the same occur in hepatic tissue (Carozzi et al., 2010). From histopathological viewpoint, SH is a progressive morphological spectrum of steatosis (Tannapfel et al., 2011). It is required at least 5% of fatty deposition within hepatocytes, ballooning of the hepatocyte and inflammation to be definitely considered SH (Tannapfel et al., 2010). In this study all histological features founded in OXA group were assigned to hepatic damage filling in the criteria diagnosis (Figure 3B). In fact, vascular sinusoidal injury appears to have an association with oxaliplatin use and it is featured by sinusoidal dilatation and erythrocyte congestion accompanied perisinusoidal fibrosis and fibrotic venular occlusion. Similar to the results depicted in this study, other studies show tht histological changes affect mainly the liver parenchyma present in vicinity of perivenular regions (Hubscher, 2006).

All compounds used herein were administered daily during 42 days in order to assess the ability of diminishing oxaliplatin-induced toxicity. Additionally, the treatments with RSV, RUT, QT or NQT were effective in preventing this damage (Figure 3A, 3C-E and G-H). A previous study from Ganapathy et al. (2010) reported resveratrol anti-inflammatory properties through the inhibition of cyclooxygenase-2 transcription. Additionally, beneficial effects of resveratrol against steatosis and alcoholic-induced hepatic lesions have been reported in rat liver, suggesting that such effect is associated to a positive regulation SIRT-1 and AMPK (Lin et al., 2006). Corroborating with our data, recent study showed that quercetin was able to decrease liver toxicity in mice with non-alcoholic SH (Marcolin et al., 2013).

Myeloperoxidase activity shows the presence of leukocytes in inflammatory processes and it is used as a marker for acute and chronic inflammatory diseases (Rensen et al., 2012). MPO is highly expressed in promyelocytes, its expression decreases as cells differentiate into granulocytic and monocytic lineages (Klebanoff, 2005). Recent study indicates oxidative stress plus inflammatory components as important factors in the development of oxaliplatin-associated hepatotoxicity (Rezazadeh et al., 2012). In our study, the animals receiving oxaliplatin exhibited an elevated MPO activity, indicating a progressive inflammatory process in the hepatic tissue, what was elucidated by the histological findings of OXA group. In fact, MPO enzyme is part of the content of neutrophils granules, and it is also involved in the production of reactive oxygen species (ROS) when interacting with hydrogen peroxide and  $\text{Cl}^-$  ions, and in inflammatory process (van der Veen et al., 2009). Conversely, animals treated with QT and NQT showed lower levels of MPO, representing a



decreased neutrophil infiltration and therefore indirectly indicating a reduction of the inflammatory process, contributing to the decreased tissue damage seen in liver histopathological analysis.

In summary, the data presented in this study shed new lights on the positive effects of these compounds in diminishing the adverse effects drawn out by oxaliplatin. Our results indicate the use of compounds such as RSV, RU, QT and NQT as effective contributors to avoid oxaliplatin-inducing hepato and neurotoxicity in a rodent model.

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**Table 1. Quantitative parameters obtained from liver tissues for histopathological examination**

Groups	Area (Sum)	Density	Roundness
Saline (10 ml/kg/week i.p., for 6 weeks)			
Saline	996154 (± 9450.9)	123.66 (± 3.58)	2.53 (± 1.76)
Oxaliplatin (10 mg/kg/week i.p., for 6 weeks)			
Saline	864314 (± 6528.7) <sup>***</sup>	106.76 (± 3.37) <sup>***</sup>	2.61 (± 3.20)
NAC	1061611 (± 9600.6) <sup>###</sup>	120.44 (± 3.05) <sup>###</sup>	2.63 (± 2.26)
RSV	1000468 (± 10789.3) <sup>###</sup>	124.33 (± 3.28) <sup>###</sup>	2.66 (± 2.54)
RUT	916160 (± 9335.2) <sup>##</sup>	122.64 (± 2.54) <sup>###</sup>	2.52 (± 1.91)
WN	892193 (± 6630.9)	113.57 (± 3.65) <sup>#</sup>	2.58 (± 2.40)
NQT	966363 (± 8544.5) <sup>###</sup>	127.20 (± 2.83) <sup>###</sup>	2.80 (± 2.64)
QT	992937 (± 8234.3) <sup>###</sup>	120.03 (± 2.79) <sup>###</sup>	2.66 (± 2.47)

All quantitative parameters were obtained from images of histological liver slides (10 images for each liver, n=5/group; imaged in 400X and counted in spatial calibration of 400 µm) using Image pro-Plus 4.5.1 software and are represented as mean ±SEM. (P < 0.001 compared with saline, # P < 0.05 compared with OXA+SAL, ## P < 0.01, and ### P < 0.0001 compared with OXA+SAL).

Abbreviations: NAC, *N*-acetylcysteine; RSV, resveratrol; RUT, rutin; WN, White nano; NQT, quercetin nanoemulsion; QT, quercetin.

## Figure Legends

**Figure 1.** Behavioral tests for assessing nociception. Von Frey filaments of different weight were used to receive a row of six responses with different filaments per animal and experiment day. A baseline of paw withdrawal threshold was measured of each group before treatment. (A) cumulative paw withdrawal frequency differences among SAL and OXA group; and (B) mechanical allodynia. Two-way analysis of variance (ANOVA) was used to calculate the difference of groups compared to control at one point of time and to make a comparison within one group at different points of time. \*\*\*  $P < 0.001$  relative to control (SAL); #  $P < 0.05$ , ##  $P < 0.01$  and ###  $P < 0.001$  relative to OXA group.

**Figure 2.** Body weight gain variation in the course of study (A), and liver weight at the end of the study (B). Data represent mean  $\pm$  SEM. One-way ANOVA, \*  $P < 0.05$  and \*\*\*  $P < 0.0001$  relative to control (SAL); #  $P < 0.05$  and ###  $P < 0.001$  relative to OXA group.

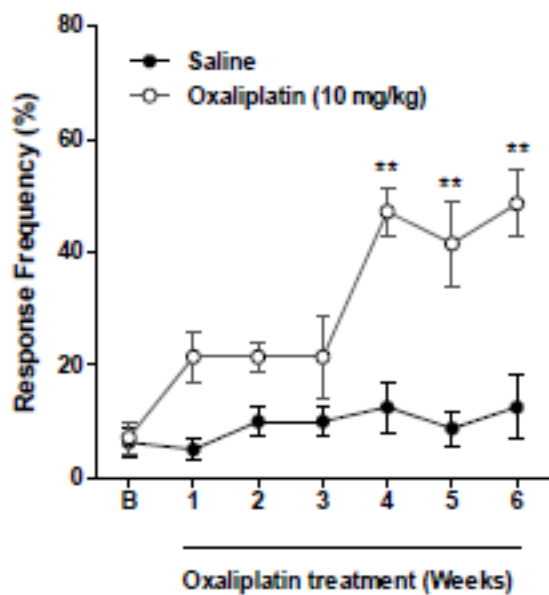
**Figure 3.** Representative images of histological evaluation of liver tissue stained with hematoxylin and eosin. (A) SAL, (B) OXA, (C) NAC, (D) RSV, (E) RU, (F) WN, (G) QT and (H) NQT groups. 400X magnification.

**Figure 4.** Effect of antioxidant compounds in myeloperoxidase (MPO) activity in the liver. Each column represents the mean of 5 animals, and the vertical lines show the SEM. \*  $P < 0.05$  relative to control (SAL). ##  $P < 0.01$  relative to OXA.

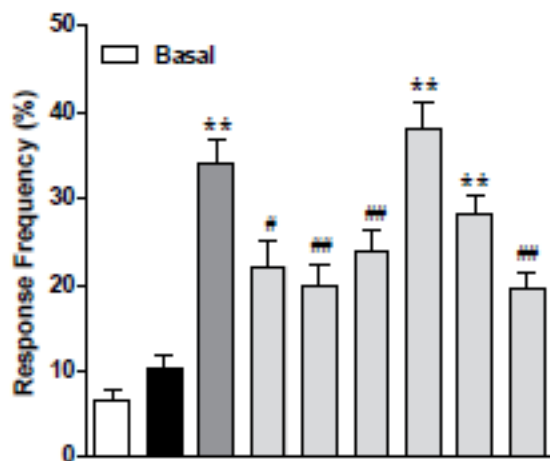
**Figure 5.** Transaminases and ALT (A) AST (B) levels were determined from blood serum. Data represent mean  $\pm$  SEM. One-way ANOVA.

Fig.1.

**A**

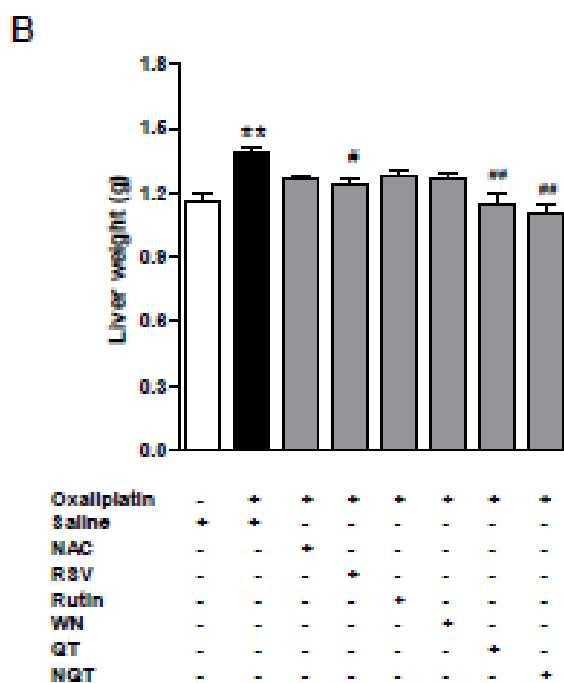
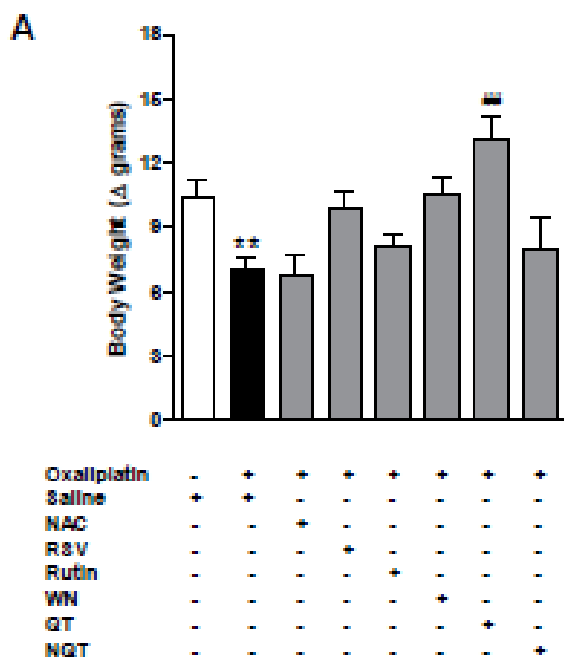


**B**



Oxaliplatin	-	-	+	+	+	+	+	+	+
Saline	-	+	+	-	-	-	-	-	-
NAC	-	-	-	+	-	-	-	-	-
RSV	-	-	-	-	+	-	-	-	-
Rutin	-	-	-	-	-	+	-	-	-
VN	-	-	-	-	-	-	+	-	-
QT	-	-	-	-	-	-	-	+	-
NQT	-	-	-	-	-	-	-	-	+

Fig.2.



**Fig.3.**

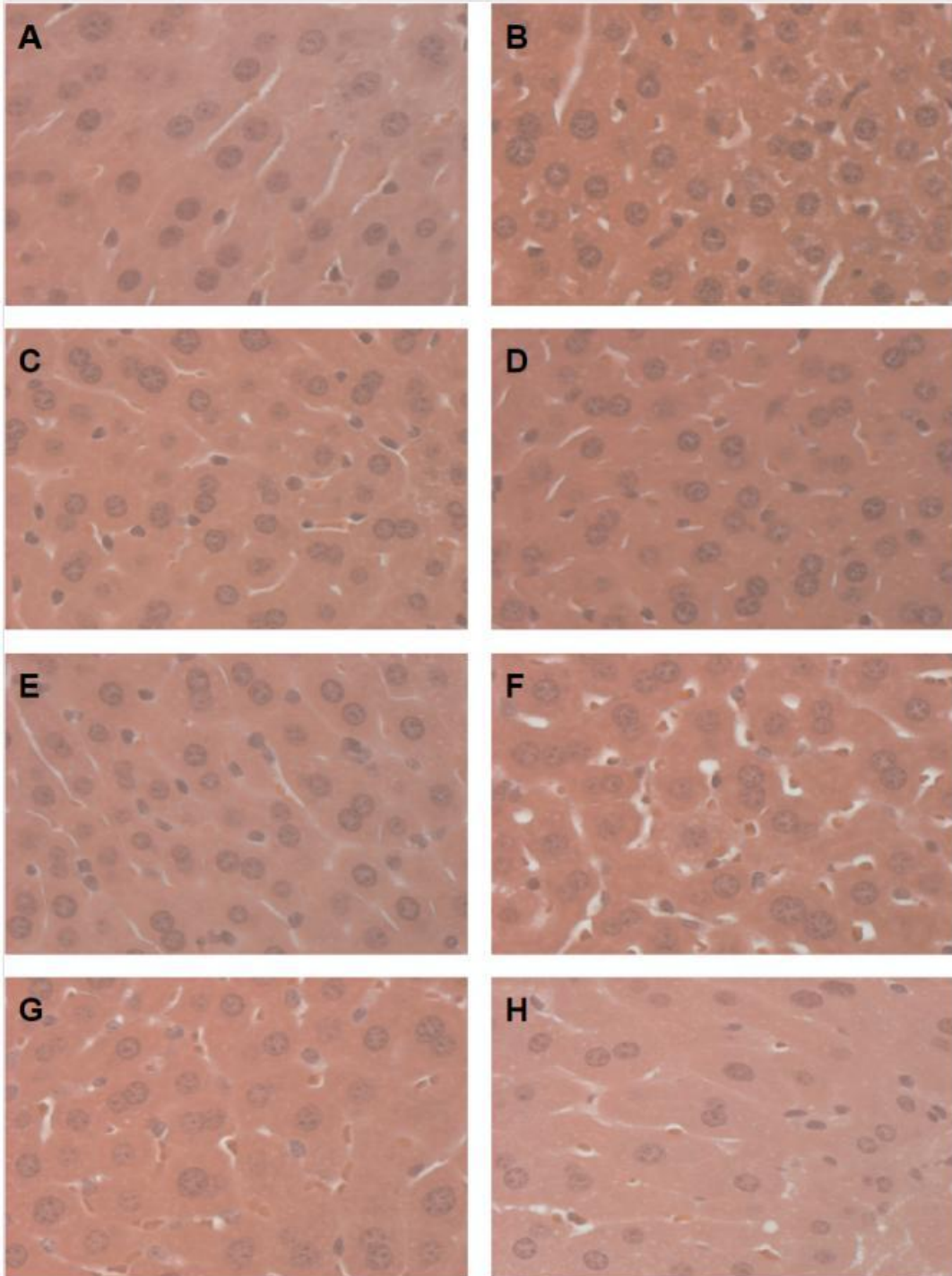
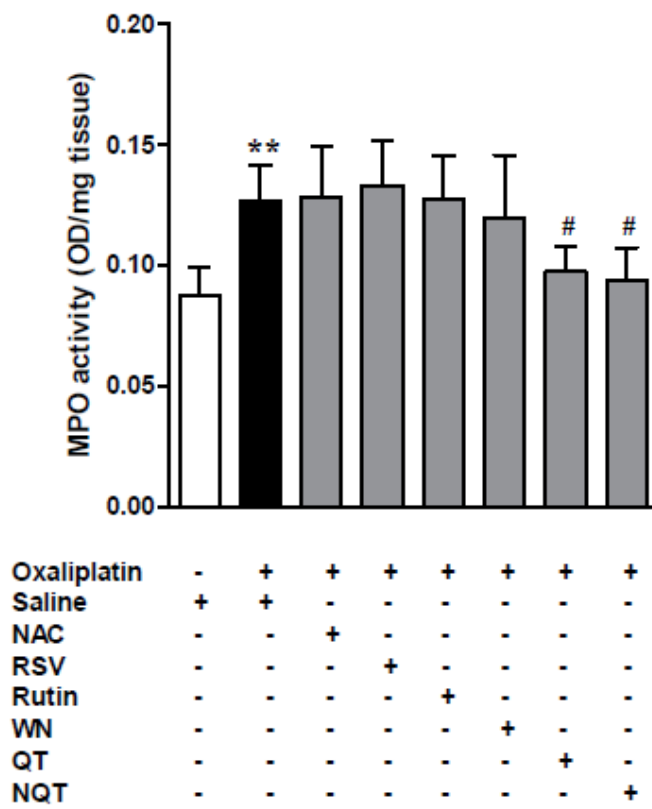
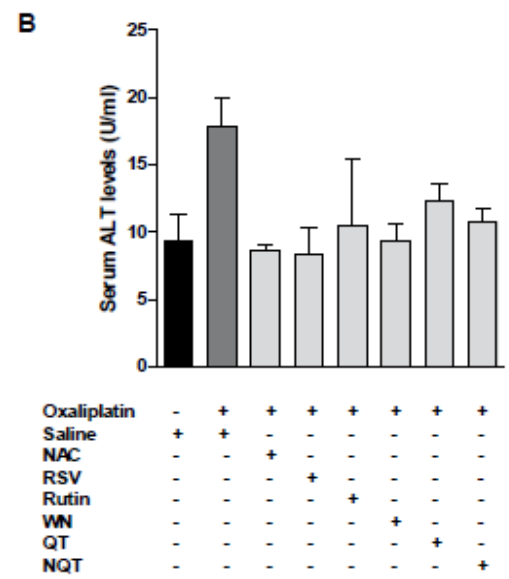
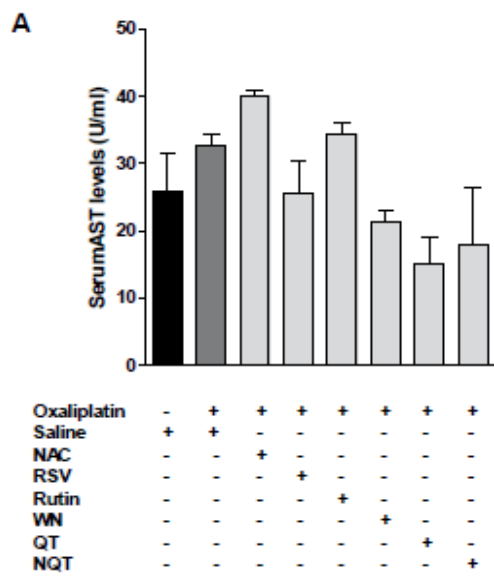




Fig.4.



**Fig. 5**



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#### 4. CONSIDERAÇÕES FINAIS

O câncer é uma doença crônico-degenerativa com impacto econômico significativo para a sociedade e, atualmente, vem sendo tratado como um grande problema de saúde pública (Caponero *et al.*, 2007). Novas terapias têm sido descobertas e vêm contribuindo muito para a qualidade e sobrevida dos pacientes, mas os estes ainda sofrem com vários efeitos colaterais dos fármacos (Authier *et al.*, 2009). Tais efeitos são decorrentes, na sua maioria, da ação dos antineoplásicos, e podem levar à interrupção do tratamento e/ou redução de dose (Sonis *et al.*, 2004). A falha na adesão ao tratamento por parte do paciente se deve principalmente à ação não seletiva dos medicamentos utilizados para tratar o câncer, os quais atingem também as células normais, comprometendo assim a eficácia da terapia (Bittencourt *et al.*, 2004).

Dentre as classes farmacológicas mais utilizadas, destacam-se os compostos da platina, os quais são muito prescritos para o tratamento de tumores sólidos humanos. Pertencente a este grupo está a oxaliplatina, fármaco de terceira geração, com estrutura similar à cisplatina (Argyriou *et al.*, 2008), e que possui efeito citotóxico semelhante aos demais análogos da platina (Foltinová *et al.*, 2009).

Deste modo, o presente estudo avaliou os efeitos de alguns compostos antioxidantes como resveratrol, rutina, quercetina e quercetina em nanoemulsão, sobre a esteato-hepatite e neurotoxicidade induzida pela oxaliplatina em camundongos Balb/c. Inicialmente realizou-se a padronização do modelo de neuro-hepatotoxicidade induzida pelo quimioterápico em camundongos, o qual se mostrou adequado apresentando as principais



características clínicas encontradas na neuropatia e na esteato-hepatite induzida pela oxaliplatina.

Na primeira etapa do presente estudo constatou-se que os animais apresentaram sintomas de neuropatia peculiar ao uso da oxaliplatina. Os resultados obtidos mostraram que os compostos antioxidantes resveratrol, quercetina em nanoemulsão e rutina foram capazes de reverter a alodinia mecânica induzida por oxaliplatina. De modo muito interessante o resveratrol e a quercetina em nanoemulsão inibiram a alodínia mecânica em maior intensidade. Corroborando com nossos dados, um estudo recente demonstrou que nos primeiros estágios da neuropatia aguda induzida pela oxaliplatina, as fibras nociceptivas periféricas são lesadas pelo estresse oxidativo e que substâncias antioxidantes são capazes de prevenir este efeito (Joseph *et al.*, 2008).

De fato, a neurotoxicidade causada pela oxaliplatina pode apresentar-se de duas formas distintas: aguda ou crônica, com sintomas iniciais de parestesias distais e periorais, tais como câimbras, espasmos tetânicos, náuseas, vômitos, entre outros (Kiernam *et al.*, 2007). Por outro lado, a neuropatia crônica é caracterizada pela perda da função motora e sensorial, semelhante ao sintoma neurológico gerado pela cisplatina, ou após a administração de oxaliplatina, a longo prazo (Deuis *et al.*, 2013; McWhinney *et al.*, 2009). Doses cumulativas podem levar à ataxia sensitiva e comprometimento funcional, levando à limitação nas atividades de vida diárias do paciente (Park *et al.*, 2009). E a toxicidade crônica pode progredir mesmo após a descontinuação do tratamento (Sprowl *et al.*, 2013).

Um segundo achado importante deste estudo foi demonstrado através do efeito tóxico no fígado, associado à esteato-hepatite induzida pela oxaliplatina. Em nosso modelo experimental houve indícios de dano hepático induzido pelo uso do quimioterápico, observado através da análise histológica e pela diminuição do peso dos fígados nos animais quando tratados com RSV, RT, QT e NQT. Além disso, a reversão da hepatotoxicidade quando os animais foram tratados com os compostos testados assemelha-se àquela encontrada no controle positivo, NAC. Há evidências de que o mecanismo de toxicidade hepática esteja relacionado ao estresse oxidativo prejudicando os mecanismos de defesa e iniciando um processo inflamatório, degenerativo e de fibrose (Ramirez *et al.*, 2010). O envolvimento do estresse oxidativo nesta patologia sugere que os antioxidantes possam ter efeitos benéficos no tratamento (Rezazadeh *et al.*, 2012).

Além disso, houve uma evidente tendência à diminuição dos níveis de ALT nos grupos tratados com os compostos em relação ao grupo OXA, confirmando assim a ação protetora das substâncias testadas. Resultados semelhantes foram também identificados no estudo de Kumar *et al.*, (2013) onde a quercetina apresentou uma propriedade hepatoprotora observada nos parâmetros medidos no soro de animais tratados comparativamente ao grupo induzido por cloroquina.

Com relação à análise da atividade da mieloperoxidase no fígado dos camundongos, observou-se que o grupo de animais tratados com a oxaliplatina teve um aumento nos valores de MPO em relação ao grupo SAL. Enquanto que os grupos tratados com os compostos quercetina e quercetina em nanoemulsão apresentaram valores diminuídos, proveniente da redução de

infiltrados de neutrófilos, indicando assim uma diminuição do processo inflamatório no fígado.

Analisando os resultados apenas entre os grupos quercetina e quercetina em nanoemulsão observou-se valores diminuídos no grupo da quercetina em nanoemulsão com relação à alodinia mecânica, análise de ALT e MPO quando comparados com o grupo tratado com quercetina na sua forma livre. De acordo com o estudo de Dora e colaboradores (2011), o desenvolvimento da quercetina em nanoemulsões possibilita um sistema que tenha capacidade de proteger o fármaco quanto a sua degradação, permite um controle de liberação do fármaco reduzindo os efeitos indesejáveis, além de aumentar a biodisponibilidade do fármaco pouco solúvel em água, atingindo os melhores efeitos terapêuticos. Além de outras vantagens, tais como, excelente estabilidade física, tolerância e viabilidade de produção.

Em suma, os dados obtidos no presente estudo, somados aos dados da literatura, reforçam as evidências de que os compostos antioxidantes quercetina, rutina e resveratrol podem minimizar os efeitos de neuro e hepatotoxicidade induzidos pelo tratamento com a oxaliplatina, ao menos no modelo animal aqui apresentado.

## **5 PERSPECTIVAS**

- 1) Analisar a avaliação imunohistoquímica para caspase-3 como indicativo de apoptose nos fígados tratados com os compostos quercetina, nonoquercetina, resveratrol e rutina.
- 2) Testar a avaliação imunohistoquímica para c-Fos, como indicador indireto de atividade neuronal nas amostras tratadas com os compostos quercetina, nanoquercetina, resveratrol e rutina.

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