UNIVERSIDADE FEDERAL DO PAMPA

ANA ZILDA CEOLIN COLPO

EFEITOS DOS EXTRATOS DE ERVA-MATE SOBRE DANOS OXIDATIVOS E SUA RELAÇÃO COM ANTI E CO-GENOTOXICIDADE

Uruguaiana

2017

ANA ZILDA CEOLIN COLPO

EFEITOS DOS EXTRATOS DE ERVA-MATE SOBRE DANOS OXIDATIVOS E SUA RELAÇÃO COM ANTI E CO-GENOTOXICIDADE

Tese apresentada ao Programa de Pós-graduação Stricto Sensu em Bioquímica da Universidade Federal do Pampa como requisito parcial para obtenção do grau de Doutora em Bioquímica.

Orientador: Prof. Dr. Vanderlei Folmer

Co-orientador: Prof. Dr. Abel Santamaría-Del Angel

Uruguaiana

2017

Ficha catalográfica elaborada automaticamente com os dados fornecidos
pelo(a) autor(a) através do Módulo de Biblioteca do
Sistema GURI (Gestão Unificada de Recursos Institucionais) .

C721e COLPO, ANA ZILDA CEOLIN
EFEITOS DOS EXTRATOS DE ERVA-MATE SOBRE DANOS OXIDATIVOS E SUA RELAÇÃO COM ANTI E CO-GENOTOXICIDADE / ANA ZILDA CEOLIN COLPO.
135 p.

Tese(Doutorado)-- Universidade Federal do Pampa, DOUTORADO EM BIOQUÍMICA, 2017.
"Orientação: VANDERLEI FOLMER".

1. BIOQUÍMICA. 2. ANTIOXIDANTE. 3. Drosophila melanogaster. 4. CÉREBRO. 5. SMART. I. Título.

ANA ZILDA CEOLIN COLPO

EFEITOS DOS EXTRATOS DE ERVA-MATE SOBRE DANOS OXIDATIVOS E SUA RELAÇÃO COM ANTI E CO-GENOTOXICIDADE

Tese apresentada ao Programa de Pós-graduação Stricto Sensu em Bioquímica da Universidade Federal do Pampa como requisito parcial para obtenção do grau de Doutora em Bioquímica.

Área de concentração: Química e Bioquímica de Produtos Biologicamente Ativos

Tese defendida e aprovada em: 9 de agosto de 2017.

Banca examinadora:

Prof. Dr. Vanderlei Folmer
Orientador
(UNIPAMPA)

Profa. Dra. Sonia Galván Arzate
(INNN)

Prof. Dr. Sandro Moreira Tuerlinckx
(URCAMP)

Prof. Dr. Elton Luís Gasparotto Denardin
(UNIPAMPA)

Prof. Dr. Rodrigo de Souza Balk
(UNIPAMPA)

Há um momento em que todos os obstáculos são derrubados, todos os conflitos se apartam e à pessoa ocorrem coisas que não tinha sonhado...

Gabriel Garcia Márquez
(Cem anos de Solidão, 1967)

RESUMO

O consumo de bebidas a base de erva-mate é parte dos hábitos e costumes de populações do sul do Brasil, Argentina e Uruguai. Seu consumo é um símbolo cultural vinculado à sociabilidade e demonstrações de cordialidade. Aprimorar o conhecimento sobre a associação de seu consumo com a saúde humana pode levar a formulação de paradoxos de grande relevância econômica e social. Assim, esta pesquisa teve como objetivos: i) avaliar a capacidade dos extratos de erva-mate modularem o equilíbrio redox a nível sistêmico em *Drosophila melanogaster* e em nível de sistema nervoso central (SNC) em ratos; ii) observar como os extratos podem afetar eventos genotóxicos relacionados a perda da heterozigose em asas de *Drosophila melanogaster*. Dietas acrescidas de colesterol foram usadas como indutoras de danos no estudo com moscas da fruta e imobilização no estudo com animais. Para avaliar a relação do extrato com mutagênese e antimutagênese empregou-se o Teste de Mutação e Recombinação Somáticas (SMART). Além disso, no delineamento experimental deste trabalho projetou-se o uso de extratos (mates) obtidos mimetizando a forma tradicional de consumo, assim nas abordagens com moscas da fruta empregaram-se extratos obtidos por extrações sequenciais. Com animais, por limitações impostas pelo experimento, empregou-se o extrato bruto obtido de forma convencional. Nesta vertente também se incluiu a avaliação dos efeitos do composto majoritário em extratos de erva-mate, o ácido clorogênico (CGA). Os resultados demostram que extratos de erva-mate, em *Drosophila melanogaster* expostas à dieta hipercolesterolêmica, são capazes de aumentar significativamente a capacidade de resistir a estresse induzido melhorando a homeostase redox. O que foi constatado pela redução dos níveis de produtos da peroxidação lipídica, recuperação da atividade enzimática da glutationa S-transferase (GST) e aumento do tempo de vida dos insetos. Nas regiões cerebrais córtex (CTX), hipocampo (HIP) e corpo estriado (STR) a indução de estresse por imobilização produz danos a lipídeos e proteínas. Mate e CGA foram capazes de atenuar esses prejuízos, demostrando seu potencial de melhorar a resistência a estresse induzido, efeito possivelmente mediado por sua capacidade antioxidante. Para completar, os resultados obtidos para a atividade genotóxica mostraram que mate não induz danos em asas de *Drosophila*, quer seja por processos mutacionais ou recombinatórios. Dados relativos à avaliação da antigenotoxicidade indicaram que os extratos reduziram a frequência de manchas mutantes em co-tratamento. No entanto, podem apresentar efeitos genotóxicos quando administrados após danos, isto pode ser resultado de sua ação na modulação de funções detoxificantes no organismo, relacionadas principalmente ao citocromo P450 (CYP). Com

base em dados da literatura estas informações levam a suposição que compostos fenólicos podem provocar inibição do CYP reduzindo a metabolização e a eliminação de drogas, o que produz acumulação e aumento da toxicidade. Conclui-se, por meio do uso dos diferentes modelos experimentais e das propostas deste estudo, que a erva-mate possui significativa atividade antioxidante e não produz efeito genotóxico *per se*, mas sua relação com antigenotoxidade e metabolização de xenobióticos precisa ser confirmada por estudos aprofundados nesta abordagem.

Palavras-chave: *Ilex paraguariensis*, ácido clorogênico, *Drosophila melanogaster*, doenças crônicas, SMART

ABSTRACT

The consumption of yerba-mate based beverages is part of the habits of the population from South Brazil, Argentina and Uruguay. Its consumption is a cultural symbol linked to sociability and demonstrations of cordiality. Improving the knowledge about the association of yerba-mate consumption with human health can lead to the creation of great economic and social relevant paradoxes. Thus, this research had as objectives: i) to evaluate the capacity of yerba-mate extracts on modulating the redox balance at systemic level in *Drosophila melanogaster* and at the central nervous system (CNS) in rats; ii) to observe how the extracts can affect genotoxic events related to heterozygosity loss on *Drosophila melanogaster* wings. Diets with high cholesterol added were used as damage inducers on the study with fruit flies and immobilization with animals. The Somatic Mutation and Recombination Test (SMART) was used to evaluate the relation of the extract with mutagenesis and antimutagenesis. In addition, in the experimental design of this work, were used extracts (mates) obtained mimicking the traditional form of consumption. Thus, in the approaches with fruit flies, extracts obtained by sequential extractions were used. Because of the limitations imposed by the experiment, the crude extract obtained by the conventional manner was used in the animals. In this strand, the evaluation of the effects of the major compound in yerba-mate extracts, the chlorogenic acid (CGA), was also included. The results showed that yerba-mate extracts, in *Drosophila melanogaster* exposed to hypercholesterolemic diet, are able to increase significantly the capacity to resist the induced stress, improving the redox homeostasis. This was evidenced by the reduction of the levels of lipid peroxidation products, glutathione S-transferase (GST) activity recovery and increase of the insects' lifespan. In the brain regions: cortex (CTX), hippocampus (HIP) and striatum (STR), the induction of immobilization stress produces damage to lipids and proteins. Mate and CGA were able to mitigate these damages, demonstrating their potential to improve induced stress resistance. This effect was possibly mediated by its antioxidant capacity. To complete, the results obtained for the genotoxic activity showed that mate does not induce damage in *Drosophila* wings, either by mutational or recombinant processes. Data for the evaluation of the antigenotoxicity indicated that the extracts reduced the frequency of mutant stains in co-treatment. However, they can present genotoxic effects when given after damage. This may be due to its action on modulating detoxifying functions in the organism, related mainly to the P450 cytochrome (CYP). Based on the literature data, this information lead to the assumption that phenolic compounds may cause CYP inhibition, reducing metabolization and drug

elimination, which produces accumulation and increased toxicity. Were possible to conclude, by using different experimental models and the study proposals, that yerba-mate has significant antioxidant activity and does not produce genotoxic effect *per se*, but its relation with antigenotoxicity and xenobiotic metabolization needs to be confirmed by deepened studies focused on this approach.

Keywords: *Ilex paraguariensis*, Chlorogenic acid, *Drosophila melanogaster*, chronic diseases, SMART

LISTA DE ILUSTRAÇÕES

SUMÁRIO

APRESENTAÇÃO

As informações aqui contidas descrevem a organização textual deste estudo. Os resultados que fazem parte desta tese estão apresentados sob a forma de dois **artigos** e um **manuscrito científico**. A formatação destas produções está de acordo com as normas de submissão dos periódicos onde os mesmos estão vinculados. Os artigos e o manuscrito representam a pesquisa na íntegra e em cada um encontram-se os resultados obtidos, materiais e métodos utilizados, a discussão dos resultados e as referências bibliográficas.

Nos itens **Introdução** e na **Revisão de Literatura** estão descritas informações a respeito dos temas projetados para o estudo. A **Discussão e Conclusões** explanam interpretações, comentários gerais e perspectivas sobre todos os resultados reportados. As **Referências Bibliográficas** contemplam apenas as citações apresentadas na **Introdução**, **Revisão Bibliográfica**, **Discussão e Conclusões** desta tese.

1 INTRODUÇÃO

Erva-mate, o produto obtido por meio do processamento de galhos e folhas da árvore *Ilex paraguariensis* St. Hill., é consumida por grande parte da população da América do Sul. No sul do Brasil, Argentina e Uruguai "mate" ou "chimarrão" são os nomes dados à bebida produzida pela infusão da erva-mate com água quente. Essa é preparada em uma cabaça seca conhecida como "cuia" e sorvida com uma espécie de canudo de metal chamado "bomba". Mais de 70% da população masculina nos estados do Rio Grande do Sul, Santa Catarina e Paraná bebem diariamente o chimarrão (BRACESCO et al., 2011).

Em extratos a base de erva-mate demostrou-se a presença de ácido clorogênico, teobromina, cafeína e ácido cafeico como compostos majoritários (COLPO et al., 2016). Independentemente da maneira de obtenção da infusão, a exposição de organismos vivos ao extrato bruto ou a seus compostos isolados tem apresentado resultados na supressão de peso, na sinalização de vias inflamatórias, em respostas citotóxicas, na manutenção do status redox e muito outros efeitos, a maioria deles atribuídos a sua significante capacidade antioxidante (SOTO VACA et al., 2012; MUÑOZ-CULLA et al., 2016; CITTADINI et al.; 2015). O interesse científico pela exploração de compostos bioativos presentes na erva-mate e efeitos associados ao seu consumo na saúde humana refletem sua importância cultural e econômica.

Bioensaios com diferentes objetivos destacam o uso da *Drosophila melanogaster* como um bom modelo para descrever relações metabólicas e genéticas, influenciadas por compostos naturais. As moscas de frutas têm funções regulatórias similares aos mamíferos, incluindo a capacidade de manter a homeostase da glicose, armazenar e mobilizar energia e modular a ingestão de alimentos (TRINH e BOULIANNE, 2013). Além disso, possuem similaridades bioquímicas e genéticas que permitem a detecção de mutações e/ou recombinações somáticas, sendo consideradas adequadas para estudar genotoxidade e seus mecanismos moleculares (GRAF et al., 1984).

Drosophila melanogaster é um dos organismos selecionados como alternativa para uso de animais de laboratório e segue a estratégia dos 3 Rs "*reduction, refinement and replacement"* (RANGANATHA e KUPPAST, 2012; DOKE e DHAWAL, 2015). Organismos de maior complexidade, contudo, mantém seu pragmatismo evolutivo e são requeridos para estudos onde se associam respostas sistêmicas e comportamentais.

Nos parágrafos anteriores foram brevemente explanados efeitos comprovados dos extratos de erva-mate. Neste contexto, o desenvolvimento deste estudo se justifica pelo interesse em expandir estes conhecimentos para responder questões ainda pouco exploradas, como suas ações na redução de supressão de efeitos negativos do consumo de uma dieta

hipercolesterêmica e sua capacidade de induzir ou prevenir eventos genotóxicos. O status redox mediado pelos extratos é o principal mecanismo a ser observado.

A ampliação dessa perspectiva para avaliação das ações dos extratos em nível de SNC de mamíferos fundamenta-se no fato de que o cérebro é particularmente vulnerável a dano oxidativo e a utilização de compostos com potencial antioxidante pode ser uma alternativa na prevenção do desenvolvimento de doenças neurodegenerativas.

2 REVISÃO BIBLIOGRÁFICA

2.1 Erva-mate

A erva-mate já era usada pelos índios Guaranis antes da chegada dos europeus na América, no entanto de acordo com o historiador Fredericindo Marés de Souza (1969) a origem do chimarrão data de mil anos antes de Cristo. Seus primeiros achados foram no Peru, onde ela era moída com outros objetos em oferendas funerárias. Quando em 1920, no Paraguai, o botânico francês August de Saint-Hilaire teve o primeiro contato com a planta, atribui-lhe o nome científico de *Ilex paraguariensis* (SANTOS, s/d).

A espécie *paraguariensis* é natural da América do Sul e ocorre no Brasil, Paraguai e Argentina, todavia, mais de 80% do total de ervais nativos situam-se no bioma brasileiro Mata Atlântica (CHECHI e SCHULTZ, 2016). Sua importância econômica é histórica, já no século XVI a produção de erva-mate era a atividade que mais se destacava na região correspondente ao Paraná. Em nível mundial, sua produção está presente no Brasil, na Argentina e no Paraguai. O Uruguai não possui ervais nativos em seu território, mas é o país que tem o maior consumo per capita, sendo de 6-8 kg/pessoa/ano, em segundo lugar está a Argentina com média de 5 kg/pessoa/ano (BRACESCO et al., 2011). A **figura 1 (a, b e c)** ilustra a árvore nativa *Ilex paraguariensis* e a preparação industrial que utiliza suas folhas e galhos, denominada erva-mate.

Na região da fronteira Brasil, Argentina e Uruguai pessoas de diferentes idades, classes sociais e estilos de vida tem o consumo do mate como parte de seus hábitos e costumes (COLPO et al., 2016). A prática de tomar mate segue alguns rituais muito particulares, envolve a questão de troca e sociabilidade, reforçando o momento de partilha e de laços sociais (DURAYSKI, 2013). Na Argentina, o mate é um dos símbolos nacionais. Neste país uma campanha trás a emblemática mensagem “En el mate estamos todos...todo está en el mate” (INYM, 2017).

No que diz respeito aos efeitos do consumo do mate na saúde, jornais de circulação regional, sites e programas de televisão tem informado a população sobre estudos que estão ampliando este conhecimento. São tomadas como exemplos algumas reportagens: o Globo Repórter (Rede Globo de televisão/Brasil) divulgou em 2012, que o mate pode combater o colesterol ruim, o diabetes e emagrecer. O jornal Minuano (Bagé/RS) em 2016 apresentou resultados parciais desta tese, apontando a redução do colesterol e o aumento do tempo de vida de moscas da fruta como um importante desfecho do consumo do mate. O jornal Uruguaio El Observador, em 2013 destacou que o mate tem efeito antioxidante e características antimutagênicas.

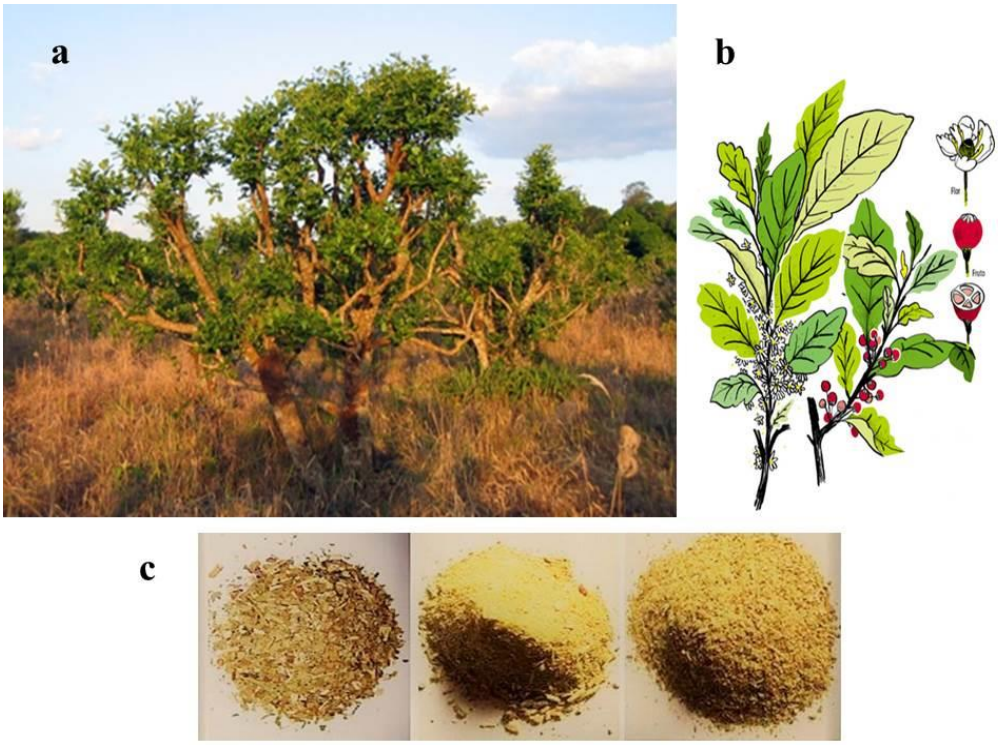


Figura 1- Erva-mate. **(a)** Árvore nativa *Ilex paraguariensis.* https://pt.wikipedia.org/wiki/Erva-mate. Consultado em 27/02/2017); **(b)** Ilustração da planta erva-mate com flor e fruto (http://www.gazetadopovo.com.br/vida-e-cidadania/especiais/erva-mate/origens.jpp. Consultado em 24/06/2017); **(c)** Amostras de erva-mate comercializadas na Argentina, Brasil e Uruguai (COLPO, 2012).

Em termos científicos, os estudos envolvendo produtos que tem como matéria prima folhas e galhos de *Ilex paraguariensis* demonstram que os principais compostos em extratos de erva-mate são ácidos fenólicos, saponinas, xantinas, minerais e vitaminas. Crescentes evidências associam a capacidade antioxidante da erva-mate ao teor destes compostos em seus extratos (HECK e MEJIA, 2007; BRACESCO et al., 2011; COLPO et al., 2016).

Com relação aos efeitos biológicos da planta, *in vitro* Colpo et al. (2016) demostraram importante capacidade "scavenger'' de radicais livres e metais. Pereira et al. (2017) utilizando ratos, concluíram que o mate foi capaz de minimizar danos oxidativos durante a menopausa, através da modulação dos mecanismos de defesa antioxidante. Além disso, tem sido comprovada sua potencialidade para interferir em vias inflamatórias (SOTO VACA et al., 2012); mediar vias de sinalização em processos citotóxicos (MUÑOZ-CULLA et al., 2016); inibir a formação de produtos finais de glicação avançada (AGE) (BAINS e GUGLIUCCI, 2017); melhorar o status diabético, aumentando a tolerância à glicose e o glicogênio hepático (PEREIRA et al., 2012). Em nível de sistema nervoso central (SNC) foi demonstrada sua capacidade ansiolítica e discinética (COLPO et al., 2007). Sua potencialidade de reduzir os

níveis de espécies reativas tem sido associada, ainda, ao aumento do tempo de vida de organismos como o nematoide *Caenorhabditis elegans* (LIMA et al., 2014).

2.1.1 Compostos bioativos nos extratos de erva-mate

A constituição química da erva-mate e de extratos obtidos a partir dela tem sido alvo de muitas investigações. Sua composição é fundamental para promover efeito antioxidante. No entanto condições como: fases do processo industrial do preparo da erva (ISOLABELLA et al., 2010), circunstâncias que a planta é cultivada (CARDOZO et al., 2007), forma de extração (MEINHART et al., 2010), e outros fatores podem afetar o teor de compostos nos extratos.

Produtos a base de *Ilex paraguariensis* são especialmente ricos em metabólitos, minerais e vitaminas (BASTOS et al., 2007; HECK e MEJIA, 2007; SUGIMOTO, 2009; BRACESCO et al., 2011). Os alcaloides como metilxantinas e terpenos, e os compostos fenólicos que são os taninos, os flavonoides e os ácidos fenólicos são os metabólitos mais comumente descritos (KHAN E MUKHTAR, 2007). Colpo et al. (2016) utilizando uma metodologia de extração que mimetiza o tradicional consumo do mate mostraram que os principais compostos obtidos em extrações sequenciais são: ácido clorogênico, ácido cafeico, cafeína e teobromina.

O ácido clorogênico (CGA) é caracterizado como um dos principais responsáveis pelos efeitos positivos associados ao consumo do mate, este corresponde a 42% dos compostos extraídos durante o consumo do chimarrão (BRACESCO et al., 2011; ARÇARI et al., 2013; SCHINELLA et al., 2014). Sabe-se que CGA é um polifenol com ampla distribuição na dieta humana, no entanto entre as bebidas e chás populares os extratos de erva-mate são os que possuem as mais altas concentrações deste composto (HEITMAN e INGRAN, 2016; BAINS e GUGLIUCCI, 2017).

CGAs são ácidos fenólicos com resíduos aromáticos que são derivados da esterificação de ácidos cinâmicos, incluindo ácidos cafeico, ferúlico e p-cumárico com ácido quínico (LIANG e KITTS, 2015). Sua forma disponível comercialmente é o ácido 5-cafeoilquínico (5-CQA) (SANTOS et al., 2010). Os benefícios dos CGAs para saúde são atribuídos à capacidade de redução do estresse oxidativo e, consequentemente, efeitos adversos relacionados com desequilíbrios no estado redox intracelular, incluindo ações cardioprotetoras, anti-tumorais e neuroprotetoras (LIANG e KITTS, 2015; HALL et al., 2015). Na **Figura 2** estão representadas as estruturas químicas dos principais CGAs.

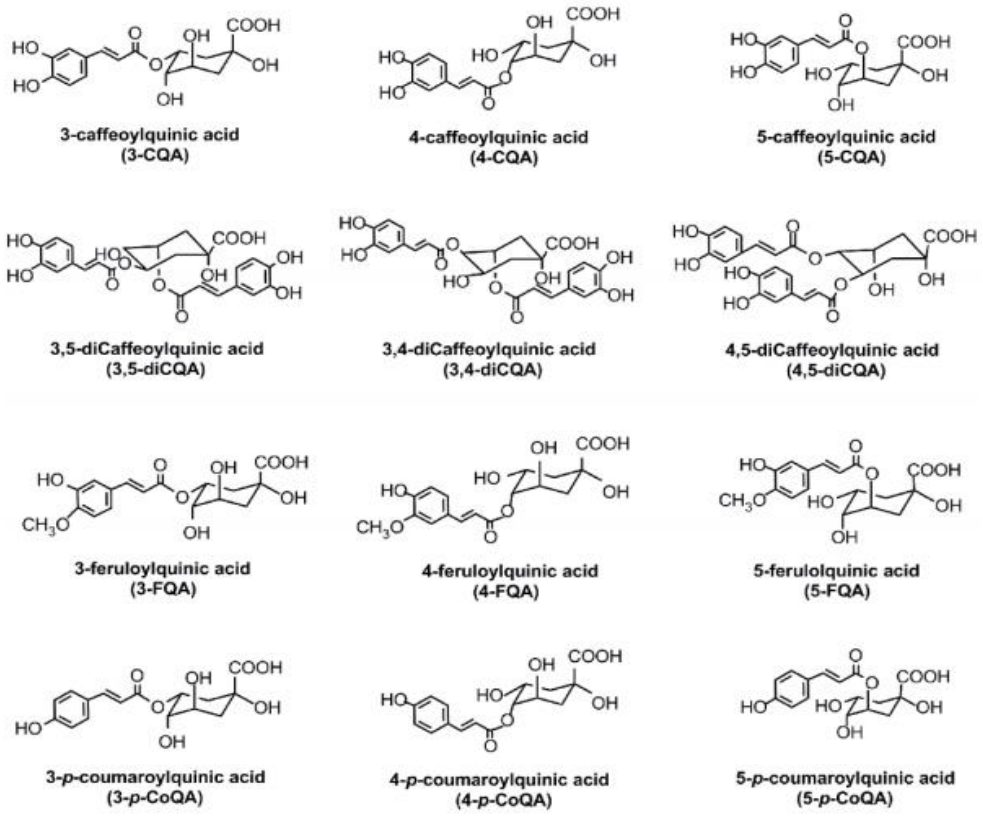


Figura 2- Estruturas químicas dos principais CGAs. 3-CQA, 4-CQA, 5-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, 3-FQA, 4-FQA, 5-FQA, 3-p-CoQA, 4-p-CoQA, 5-p-CoQA (LIANG e KITTS, 2015).

Além dos polifenóis e metilxantinas, saponinas presentes nos extratos também são de particular importância. A fração saponínica em erva-mate é constituída basicamente de compostos triterpênicos onde são encontrados derivados glicosilados do ácido ursólico e do ácido oleanólico (HEINZMANN e SCHENKEL, 1995). Tais substâncias são responsáveis pelo amargor e espuma do mate, além de diversas propriedades biológicas (GNOATTO et al., 2007).

As principais saponinas identificadas na erva-mate são: mate saponinas 1-5 (GOSMANN et al., 1995; GOSMANN, SCHENKEL e SELIGNMANN, 1989; KRAEMER et al., 1996). Coelho et al. (2010) apontam a predominância de matesaponinas 1, 2, e 3 na solução extrativa correspondente a erva-mate do Rio Grande do Sul. A estrutura química destes compostos é apresentada na **Figura 3**.

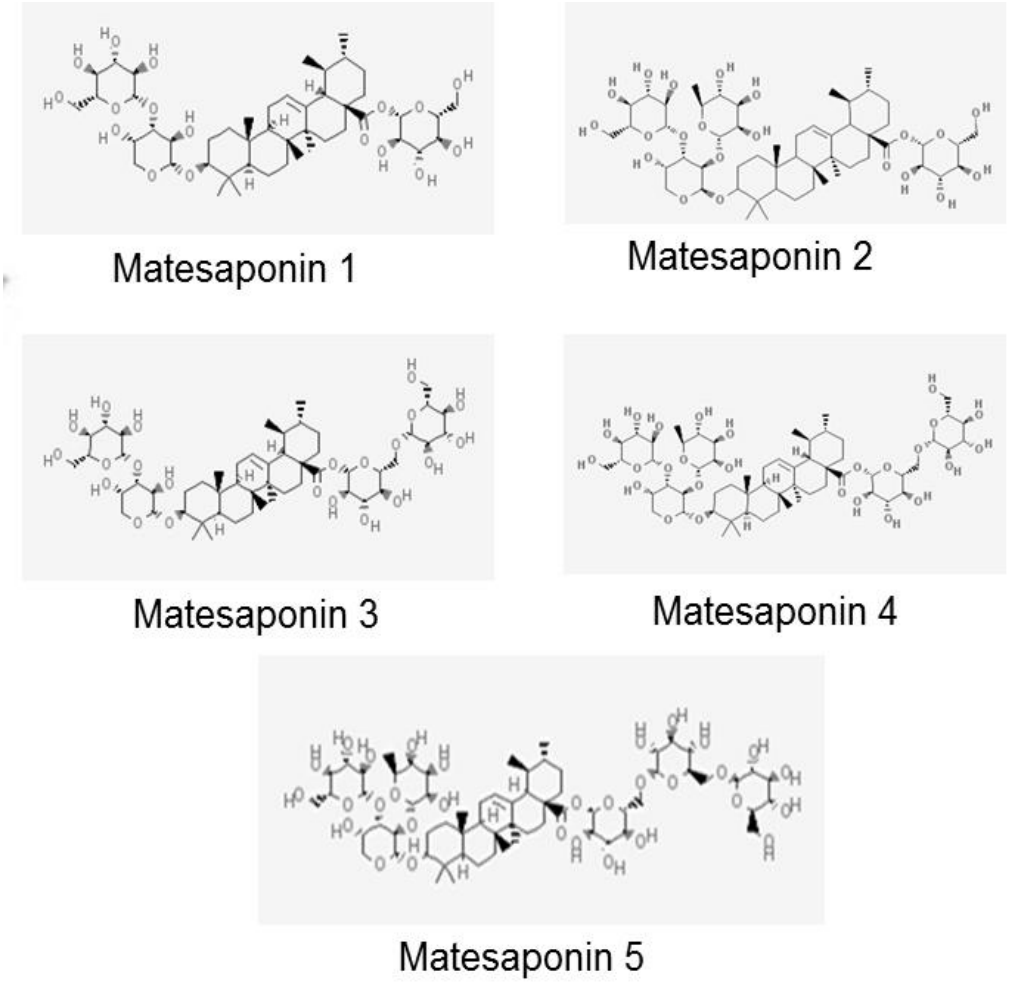


Figura 3- Estrutura química das principais matesaponinas. (GOSMANN et al., 1995; GOSMANN, SCHENKEL e SELIGNMANN, 1989; KRAEMER et al., 1996; https://www.ncbi.nlm.nih.gov/pccompound).

Efeitos benéficos de saponinas incluem atividade hipocolesterolêmica, antimutagênica e antiinflamatória (PUANGPRAPHANT et al., 2011). Uma fração de saponina purificada do mate comprovou ter ação na redução no peso de gordura visceral e na oxidação da glicose do tecido hepático e adiposo (RESENDE et al., 2012). Além disso, Puangpraphant et al. (2011 e 2013) apontaram que mate saponinas podem inibir a inflamação através da supressão da via de sinalização NF-kB e também reduzir a proliferação de células de câncer de cólon por via caspase dependente.

2.2 Erva-mate e estresse oxidativo

Estresse oxidativo compreende o conjunto de condições intra e extracelulares que levam a geração excessiva de espécies reativas (GALLEY, 2011). Durante o metabolismo basal normal das células aeróbicas existe constante formação desses radicais, entretanto, o

aumento na sua produção ou a diminuição das defesas antioxidantes podem levar a uma condição de desequilíbrio redox e induzir estresse oxidativo (HALLIWELL e GUTTERIDGE, 2007). De importância fisiológica, são descritas três principais espécies reativas de oxigênio (ROS): o aníon superóxido ($O_2^{\bullet-}$), o radical hidroxila (•OH) e o peróxido de hidrogênio (H_2O_2) (BIRBEN et al., 2012).

A principal fonte endógena de radicais livres é a mitocôndria, isto porque ela gera ATP de um modo dependente de oxigênio (HOLMSTRÖM e FINKEL, 2014). A produção de ROS pelas mitocôndrias é importante para a função celular normal e para a sobrevivência, uma vez que as mitocôndrias têm outros papéis. Além da produção de energia as mitocôndrias atuam, por exemplo, em vias de sinalização celular e na homeostase de cálcio e ferro (GALLEY,2011). Um aumento da produção de ROS neste nível pode provocar danos em proteínas, membranas e DNA (GALLEY, 2011).

Entre as fontes exógenas de produção de ROS podem ser citadas: fumaça de cigarro, exposição ao ozônio que pode causar peroxidação lipídica e induzir influxo de neutrófilos para o epitélio das vias aéreas, exposição prolongada a níveis altos de oxigênio (hiperóxia) e radiação ionizante. A radiação na presença de O_2 converte radicais hidroxil, superóxido e radicais orgânicos em peróxido de hidrogênio e hidroperóxido, além disso, leva ao acúmulo de metais pesados no organismo (BIRBEN et al., 2012; HOLMSTRÖM e FINKEL, 2014). Metais pesados possuem potencial para produzir substâncias químicas altamente reativas e causar oxidação de grupos sulfidrilo de proteínas, depleção de proteínas, danos ao DNA, peroxidação lipídica e vários outros efeitos (JAN et al., 2015). Organismos aeróbios têm sistemas antioxidantes integrados, que incluem antioxidantes enzimáticos e não-enzimáticos, estes geralmente são eficazes no bloqueio dos efeitos nocivos das espécies reativas (BIRDEN et al. 2012). A ativação de mecanismos antioxidantes é capaz de retardar ou inibir a oxidação de um substrato, podendo agir em diferentes níveis da sequência oxidativa (HALLIWELL e GUTTERIDGE, 2007).

A superóxido dismutase (SOD) é a enzima mais abundante do organismo. As células são dotadas de duas SODs principais, uma citoplasmática, que é a CuZnSOD e outra, mitocondrial, que é a MnSOD, esta contendo manganês e aquela contendo cobre-zinco na mesma molécula (HALLIWELL e GUTTERIDGE, 2007). Estas enzimas juntamente com a catalase e a glutationa peroxidase são as principais defesas antioxidantes que atuam nos organismos superiores (HALLIWELL e GUTTERIDGE, 2007). A catalase está presente no citoplasma de todos os animais, sendo seu principal papel biológico regular a concentração

intracelular de H_2O_2 no estado estacionário, o que ajuda a manter a homeostase celular (HALLIWELL e GUTTERIDGE, 2007; KRYCH-MADEJ e GEBICKA, 2017).

A glutationa é um antioxidante não proteico importante nas células e a proporção de sua forma reduzida e oxidada (GSH/GSSG) é um indicador do estado redox celular (SCHAFER e BUETTNER, 2001). O pool de glutationa (GSH) está fortemente ligado ao metabolismo da glicose através da via das pentoses fosfato, uma vez que esta via gera o NADPH que é necessário para manter GSH numa forma reduzida (HOLMSTRÖM e FINKEL, 2014). As transferases de glutationa (GSTs, também conhecidas como glutationa S-transferases) encontradas principalmente no citosol também participam dos mecanismos celulares de detoxificação, inativando metabólitos secundários, como aldeídos insaturados, epóxidos e hidroperóxidos (SHEEHAN et al., 2001; BIRDEN et al. 2012). A **figura 4** apresenta fontes endógenas e exógenas de agentes oxidantes, bem como vias de sinalização ativadas em condições de desequilíbrio.

A ligação entre metabolismo e oxidantes, tem um papel importante na homeostase redox, no entanto em condições patológicas os sistemas antioxidantes podem ser sobrecarregados. Nesse contexto o consumo de nutrientes antioxidantes auxilia na regulação redox e é capaz de aliviar a deterioração causada por ROS (WANG et al., 2015). Nutrientes antioxidantes são compostos que não podem ser produzidos no corpo e necessitam ser fornecidos através de alimentos ou suplementos (GUPTA et al., 2014).

No passado, apontou-se que antioxidantes são capazes de influenciar significativamente a progressão de doenças. Alimentos que contêm antioxidantes naturalmente, mas não ricos em calorias, ou seja, frutas, legumes e grãos, ajudam a manter a saúde e retardar o início de doenças (GUTTERIDGE e HALLIWELL, 2010). Compostos com capacidade antioxidantes reagem diretamente com os radicais livres, levando a formação de produtos menos reativos e participam na ativação de genes que codificam proteínas envolvidas no sistema enzimático antioxidante, e/ou no silenciamento de genes que podem contribuir para ocorrência de estresse oxidativo (FONSECA, 2007).

O mecanismo de ação dos antioxidantes pode ocorrer de duas maneiras: quebra cadeia ou prevenção. No processo de quebra cadeia, quando um radical liberta ou rouba um elétron, um segundo radical é formado, os antioxidantes interferem na etapa de propagação radicalar. O modo de prevenção exerce a mesma ação, no entanto ele age até que o radical formado seja estabilizado por um antioxidante quebra cadeia ou simplesmente se desintegre em um produto inofensivo (GUPTA et al., 2014).

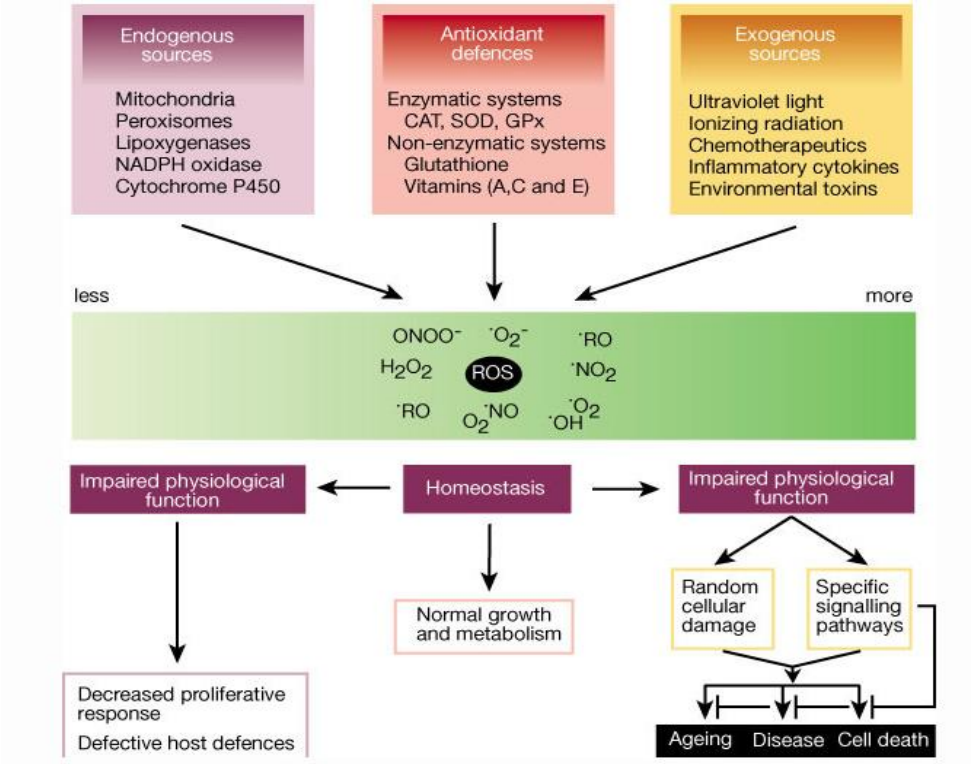


Figura 4- Oxidantes geradas como resultado do metabolismo intracelular normal nas mitocôndrias. Neste contexto, um sofisticado sistema de defesa antioxidante enzimático e não-enzimático, incluindo catalase (CAT), superóxido dismutase (SOD) e glutationa peroxidase (GPx), neutraliza e regula os níveis gerais de ROS para manter a homeostase fisiológica. A redução dos níveis de ROS abaixo do ponto de referência homeostático pode interromper o papel fisiológico dos oxidantes. Da mesma forma, aumento de ROS também pode ser prejudicial e levar à morte celular ou acelerar o envelhecimento e doenças relacionadas com a idade. ROS produz danos a proteínas, lipídios, DNA e pode ativar vias de sinalização redox-sensíveis (FINKEL e HOLBROOK, 2000).

Os polifenóis podem desempenhar papéis protetores através da transferência de um átomo de hidrogênio, transferência de um único elétron e/ou quelação de metal (LEOPOLDINI et al., 2011; COLPO et al., 2016). Quando um antioxidante destrói um radical ele se torna oxidado, portanto, os recursos antioxidantes devem ser constantemente restaurados no corpo (GUPTA et al., 2014). Deste modo extratos de erva-mate, especialmente ricos em polifenóis e outros compostos com potencial antioxidante, podem ser considerados para garantir a supressão diária de antioxidantes através da dieta.

Muitas condições podem promover danos oxidativos e pelas especificidades deste estudo se configuram como relevantes fatores associados à dieta, genotoxidade e condições físicas e ambientais que podem aumentar a produção de espécies reativas em nível de Sistema Nervoso Central (SNC), temas estes que serão abordados na sequência.

2.2.1 Dieta e estresse oxidativo

Diferentes nutrientes, tais como determinados tipos de gordura dietética parecem influenciar o desenvolvimento de doenças relacionadas à produção excessiva de ROS e/ou disfunções do sistema antioxidante. O tipo de gordura dietética afeta a estrutura funcional da mitocôndria, bem como a sua susceptibilidade ao estresse oxidativo (LÉON-GONZÁLES et al., 2015). Componentes da dieta podem influenciar estratégias de vida através de suas características energéticas e de sinalização (SKORUPA et al., 2008). Kardinaal et al. (2015) observam que o metabolismo mantém a homeostase em condições crônicas hipercalóricas ativando respostas adaptativas, que eventualmente trazem consequências negativas para a saúde, tais como armazenamento de energia.

Embora o tecido adiposo tenha sido considerado por muito tempo como um reservatório passivo de lipídeos, sabe-se agora que ele é um órgão endócrino ativo que produz uma variedade de adipocinas e é um importante regulador do metabolismo energético (OISHI e MANABE, 2016). Defeitos nos processos de armazenamento e uso da gordura dietética podem levar a mudanças nos níveis de triacilglicerol (TAG) e a uma gama de distúrbios fisiológicos, incluindo obesidade (SIEBER e THUMMEL, 2009).

A obesidade é considerada a base patológica chave para diabetes e aterosclerose, isto porque a ela associa-se uma inflamação sistêmica sustentada (OISHI e MANABE, 2016). O aumento alarmante na prevalência destes distúrbios em populações humanas tem concentrado a atenção na compreensão dos mecanismos moleculares que coordenam a absorção de nutrientes na dieta, bem como a homeostase de TAG (SIEBER e THUMMEL, 2009).

Hiperlipidemia com ou sem aterosclerose é amplamente conhecida por ser o principal fator de risco para o desenvolvimento de doenças coronarianas. Essa alteração pode causar o aumento da produção de ROS, e o estresse induzido desempenha papel importante na etiologia de doença cardíaca coronária (TANTAWY, 2015). Aumento da peroxidação lipídica e diminuição da atividade de enzimas antioxidantes produzidos por ROS são eventos iniciais em condições de hiperlipidemia em seres humanos (YANG et al., 2008). Estados hiperlipidêmicos estão associados a propriedades físicas alteradas das membranas celulares, o que pode facilitar a fuga de radicais livres da cadeia de transporte de elétrons mitocondrial ou a ativação da NADPH oxidase (YANG et al., 2008).

Além disso, estresse oxidativo nesta condição, produz disfunção na produção endógena de óxido nítrico (NO). A inativação do NO endógeno pode provocar a formação de peroxinitrito ($ONOO^-$) ou alteração das óxido nítrico sintases (NOS) com coprodução de $O_2^{\bullet-}$ (HALLIWELL e GUTTERIDGE, 2007). A formação excessiva de $ONOO^-$ está associada a

diminuições significativas do teor de glutationa celular e subsequentemente de NOS (TANG et al., 2014).

Vale ressaltar, no entanto, que o colesterol é essencial para muitos processos biológicos, ele desempenha papéis críticos na permeabilidade e fluidez das membranas celulares, na modulação da atividade de vias de transdução de sinal intracelular e também serve como precursor na síntese de hormônios esteroides (NIWA e NIWA, 2011), sendo seu metabolismo fundamental para a homeostase energética.

Sobre a ação dos extratos de erva-mate no perfil metabólico de humanos pouco se sabe, no entanto a relação entre dieta, envelhecimento, colesterol e doenças crônicas vem sendo amplamente estudada. O conhecimento dos mecanismos envolvidos na gênese de doenças influenciadas por esses fatores parece ser a chave para o desenvolvimento de terapias para prevenção de doenças cardiovasculares e prolongamento do tempo de vida saudável em humanos (OTANI et al., 2013; PENG et al., 2009; GUTTERIDGE e HALLIWELL, 2007). Neste contexto, *Drosophila melanogaster* é considerada um bom modelo para estudar a regulação do colesterol, sendo as moscas da fruta utilizadas com sucesso para a análise de mecanismos moleculares que regulam o metabolismo e a homeostase do mesmo (NIWA e NIWA, 2011; TRINH e BOULIANNE, 2013).

2.2.2 *Drosophila melanogaster* como modelo para estudar metabolismo

A disponibilidade de sequências genômicas, a facilidade de manipulação genética e a grande coleção de mutantes disponíveis fazem da *Drosophila melanogaster* um sistema atrativo que possibilitou um melhor entendimento das doenças humanas no nível molecular (BOTAS, 2007). A compreensão de como nutrientes são integrados no metabolismo, como são influenciados os mecanismos moleculares de sobrevivência e como podem ser inibidas ou ativadas vias de sinalização é facilitada neste modelo (TATAR et al., 2014).

Muitas vias metabólicas humanas são conservadas na mosca, neste inseto estão presentes homólogos que vão desde hormônios circulantes até enzimas chaves, além de vias de sinalização que controlam a detecção e a utilização de carboidratos, lipídios e aminoácidos. Por exemplo, no pâncreas as células α e β controlam os níveis de glicose no sangue através da secreção equilibrada de glucagon e insulina, respectivamente (BHARUCHA, 2009; CAMPBELL e DRUCKER, 2015).

A possibilidade das manipulações dietéticas serem relativamente fáceis e econômicas também contribui para seu uso no estudo de efeitos da supressão ou adição de algum componente nutricional. Na mosca o intestino é também o local de absorção lipídica, este é

metabolizado principalmente por um homólogo de lipase gástrica de mamíferos, o *Magro*, em monoacilglicerídeos e ácidos graxos, que podem então ser absorvidos por enterócitos, convertidos em diacilglicéridos e transportados na hemolinfa como lipoproteínas (SIEBER e THUMMEL, 2009).

Drosophila melanogaster também requer colesterol como precursor para hormônios esteroides e como componente estrutural das membranas celulares. No entanto, ela não é hábil para sintetizá-lo e deve obtê-lo da dieta, sendo os genes dos receptores nucleares essenciais para a mediação da resposta transcricional ao colesterol dietético (GILBERT et al., 2002; NIWA e NIWA, 2011).

O receptor nuclear DHR96 atua como os Receptores X (LXRs) do fígado de mamíferos, sendo o centro do controle da homeostase do colesterol nas moscas da fruta. Ele é essencial para captação, tráfico, armazenamento e transcrição de genes por colesterol mediados (HORNER et al., 2009). Genes com funções na manutenção da homeostase do colesterol são fortemente dependentes da função do DHR96, incluindo homólogos dos vertebrados NPC1L1 (*NPC1 like intracellular cholesterol transporter 1*), NPC2 (*Intracellular Cholesterol Transporter 2*) e ABCA1 (*ATP Binding Cassette Subfamily A Member 1*) (BUJOLD et al., 2010, GENESCARDS, 2010). Em moscas mutantes com DHR96 anulado, demostrou-se que a absorção de colesterol é afetada pela desregulação do *NPC1B*. Como resultado, larvas mutantes mostram acúmulo de colesterol quando expostas a uma dieta rica neste tipo de gordura e diminuição da taxa de sobrevivência quando submetidas a meios com baixo nível do mesmo (HORNER et al., 2009).

DHR96 também está envolvido no controle das respostas xenobióticas, através da regulação da expressão de enzimas de desintoxicação (KING-JONES et al., 2006). Moscas mutantes com DHR96 anulado apresentaram genes mal regulados e desajuste das funções metabólicas, o que afetaria respostas xenobióticas dentro do animal. Enzimas metabolizadoras xenobióticas, tais como citocromos P450 e UDP-glucuronosil-transferases (UGTs) consomem NADPH ou NADH e glicose e Glutationas S-tranferases (GSTs) consomem glutationa, o que denota o custo elevado da metabolização de toxinas e a necessidade do adequado suprimento (KING-JONES et al., 2006). Estudos adicionais, no entanto, adicionam a possibilidade de que fatores transcricionais suplementares também estejam envolvidos na regulação de respostas a xenobióticos (MISRA et al., 2011).

Com base nesses conhecimentos e em estudos envolvendo o uso de moscas da fruta, Tennessen et al. (2014) publicaram uma ampla revisão descrevendo os métodos desenvolvidos para estudos do metabolismo em *Drosophila*, com foco na homeostase

energética e fisiologia. Além disso, revisaram protocolos para a intervenção dietética e para quantificar metabólitos básicos no animal, e destacam o uso deste modelo para o estudo dos mecanismos que mantêm a homeostase da energia, particularmente em processos como diabetes e obesidade.

2.2.3 Estresse e danos oxidativos a nível de SNC

O cérebro dos mamíferos é particularmente vulnerável a danos oxidativos, sendo a produção de ROS no cérebro um importante fator para o desenvolvimento de processos neurodegenerativos (POPA-WAGNER, 2013). A sensibilidade deste órgão a alterações no status redox ocorre principalmente porque ele é um importante metabolizador de oxigênio. No entanto, Halliwell e Gutteridge (2007) apontam outros fatores como relevantes para o aumento da sua suscetibilidade. Entre eles está o fato que a membrana neuronal contém grande quantidade de ácidos graxos poli-insaturados, que são suscetíveis ao processo de oxidação. O 4-hidroxinonenal (4-HNE), um produto da peroxidação lipídica, é citotóxico aos neurônios, sendo capaz de aumentar os níveis da Ca^{2+}, inativar transportadores de glutamato, lesar neurofilamentos de proteínas e inativar a enzima α-cetoglutarato desidrogenase.

Além disso, muitos neurotransmissores são moléculas autooxidavéis, este processo é acelerado pela presença de $O_2\bullet^-$, e também pela passagem de íons como manganês e ferro. Dopamina, L-DOPA, serotonina e noradrenalina reagem com O_2 para gerar $O_2\bullet^-$, H_2O_2 e quinonas/semiquinonas que podem depletar GSH e se ligar a grupos SH de proteínas. Superóxido pode reagir com noradrenalina, dopamina e serotonina e iniciar sua oxidação, que continua com a produção de mais ROS. O dano neuronal produzido pode provocar a liberação de glutamato, favorecendo um ciclo vicioso de eventos.

Radicais livres também são produzidos pela ativação da micróglia, que pode gerar $O_2^{\bullet-}$, H_2O_2 e citocinas e pelo próprio metabolismo cerebral o qual fornece grande quantidade de H_2O_2, via SOD e outras enzimas como a monoamina oxidase. A ação dessas espécies reativas pode ser significante porque as defesas antioxidantes modestas. O citocromo P450 está presente apenas em algumas regiões do cérebro e os níveis de catalase são reduzidos em regiões como córtex e cerebelo. Espécies reativas podem contribuir, também, para abrir a barreira hematoencefálica permitindo a entrada de neurotoxinas.

Condições que produzem estresse psicológico também participam na geração de ROS em nível cerebral. Em resposta aos estressores, o eixo hipotálamo-hipófise-adrenal (HPA) é ativado, resultando na liberação de glicocorticoides a partir do córtex adrenal. Os estressores psicológicos ou ameaças percebidas são transmitidos através de circuitos do proencéfalo

límbico (áreas conectadas funcional e anatomicamente e que estão relacionadas com emoção, motivação e autopreservação) e levam a alterações no comportamento e reatividade fisiológica ao estresse. As regiões cerebrais envolvidas nesse circuito incluem hipocampo, amígdala, córtex, núcleo leito da estria terminal e núcleo do trato solitário (BOYLE et al., 2006; BURNS et al., 2014).

A resposta ao estresse fisiológico ocorre em duas fases. A primeira é considerada a "reação de alarme" e envolve a rápida ativação do sistema nervoso autônomo com a liberação de epinefrina e noradrenalina a partir da adrenal. Esses hormônios elevam a taxa metabólica basal, aumentando a pressão arterial, a respiração e o fluxo de sangue para os órgãos vitais. Numa fase posterior, o eixo HPA também é ativado, em uma resposta mediada por hormônios de glicocorticoides (corticosterona em roedores e cortisol em humanos) que agem como reguladores transcricionais (LUCASSEN et al., 2014).

A prolongada superprodução de glicocorticoides, como no estresse crônico, danifica estruturas cerebrais (especialmente o hipocampo) essenciais para o controle do eixo HPA (SAPOLSKY, 1986). Vyas et al. (2002) sugerem que esse tipo de estresse pode levar a desequilíbrios na função do eixo HPA através de uma perda gradual do controle inibitório do hipocampo, bem como um ganho no controle excitatório exercido pela amígdala. No hipocampo, estresse e glicocorticoides mostraram causar retração com perda das espinhas dentríticas dos neurônios (McEWEN et al., 2015). No córtex pré-frontal, essa condição produz desramificação e encolhimento dos dendritos, enquanto há expansão dos dendritos dos neurônios corticais orbitofrontais, o que provavelmente é o fator causal do aumento da vigilância observado (LISTON et al., 2006).

Morfologicamente os neurônios do SNC diferem em tamanho, número e complexidade dos dendritos, número e distância entre as conexões sinápticas, comprimento dos axônios, extensão da mielinização axonal, neurotransmissores que utilizam, entre outras características (WANG e MICHAELIS, 2010). Em função dessas diferenças as respostas ao estresse não são uniformes, sendo as regiões cerebrais mais vulneráveis as camadas superficiais do córtex cerebral, o giro dentado, o subículo (parte mais baixa do hipocampo) e o estriado dorsolateral (LINDVALL et al., 1986).

Em animais experimentais o estresse crônico induzido afeta a atividade motora, inibe o ganho de peso corporal e aumenta os níveis plasmáticos de cortisol. Em nível molecular está relacionado à diminuição da atividade de enzimas antioxidantes como a glutationa peroxidase (GPx) e a danos oxidativos a lipídios, proteínas e DNA. As causas prováveis são disfunção mitocondrial, perturbação do metabolismo energético, danos neuronais, neurogênese

prejudicada e indução de eventos de sinalização por apoptose (LIU et al., 1996; LIU et al., 2013).

Estresse por restrição é considerado eficaz para incitar estresse físico e psicológico, sendo um modelo adequado para avaliar dano oxidativo em diferentes regiões do cérebro, incluindo hipocampo, córtex cerebral, corpo estriado e, dependendo das condições empregadas, o cérebro como um todo (ZAIDI et al.,2006; SAHIN e GUMUSLU, 2007).

Sobre estas condições, a utilização de compostos com potencial antioxidante para manutenção da homeostase redox e para contrariar os eventos prejudiciais provocados pela imobilização aguda pode ser útil (MÉNDEZ-CUESTA et al., 2011; CITTADINI et al., 2015). Recentes evidências sugerem que o uso de compostos, como polifenóis pode produzir, no sistema nervoso central, efeitos benéficos que envolvem diminuição do estresse oxidativo/inflamatório conduzindo à expressão de genes que codificam enzimas antioxidantes, fatores neurotróficos e proteínas citoprotetoras (VAUZOUR, 2012).

Cittadini et al. (2015) avaliaram os efeitos da ingestão oral de extratos ricos em polifenóis na homeostase redox de regiões encefálicas de murinos. Entre os extratos estudados estava o de erva-mate, sendo verificado que este apresentou um significativo perfil redox. Os mesmos autores observam que a planta poderia ser uma fonte de agentes quimio preventivos em patologias neuro degenerativas relacionadas com estresse oxidativo.

Estudos *in vitro* indicam que flavonóides ou seus metabólitos podem cruzar a barreira hematoencefálica (BHE) com um potencial de penetração influenciada por sua lipofilicidade (YOUDIM et al., 2003; SCHEEPENS et al., 2010). Polifenóis menos polares ou metabólitos (por exemplo, derivados o-metilados) têm maior absorção do que as mais polares (SCHEEPENS et al., 2010). A biodisponibilidade destes compostos é dependente, ainda, da sua compartimentação entre o líquido cefalorraquidiano e o fluido extracelular nos diferentes tipos de células cerebrais (SCHAFFER e HALLIWELL, 2012). Para ilustrar esses registros na **Figura 5** estão retratados os processos que regem a biodisponibilidade de polifenóis no cérebro de mamíferos.

Alguns autores observam que mesmo em baixas concentrações estes compostos são capazes de exercer neuroproteção e efeitos neuromoduladores (VAUZOUR et al., 2012; REGE et al., 2014). Lin et al. (2007) demostraram que após administração oral de chá verde (100 mg/kg), aproximadamente 5% das catequinas ou seus metabólitos ficaram biodisponíveis e penetraram a BHE.

A função cerebral pode ser afetada pelos polifenóis por sua atuação fora do SNC, que pode se dar pelo incremento do fluxo sanguíneo cerebral ou pela modulação de vias de

sinalização dos órgãos periféricos para o cérebro. Isso leva a alterações no fluxo sanguíneo cerebral, aumentando a vascularização e facilitando a neurogênese, dois eventos importantes na manutenção dos desempenhos cognitivos (VAUZOUR, 2017). Dentro do SNC, podem modular mecanismos associados à neuroinflamação, através do seu potencial para intervir em vias de sinalização que controlam a ativação das células gliais, liberação de citoquinas, expressão de iNOS, produção de óxido nítrico, atividade de NADPH oxidase e também em rotas que determinam a apoptose neuronal (SPENCER et al., 2012).

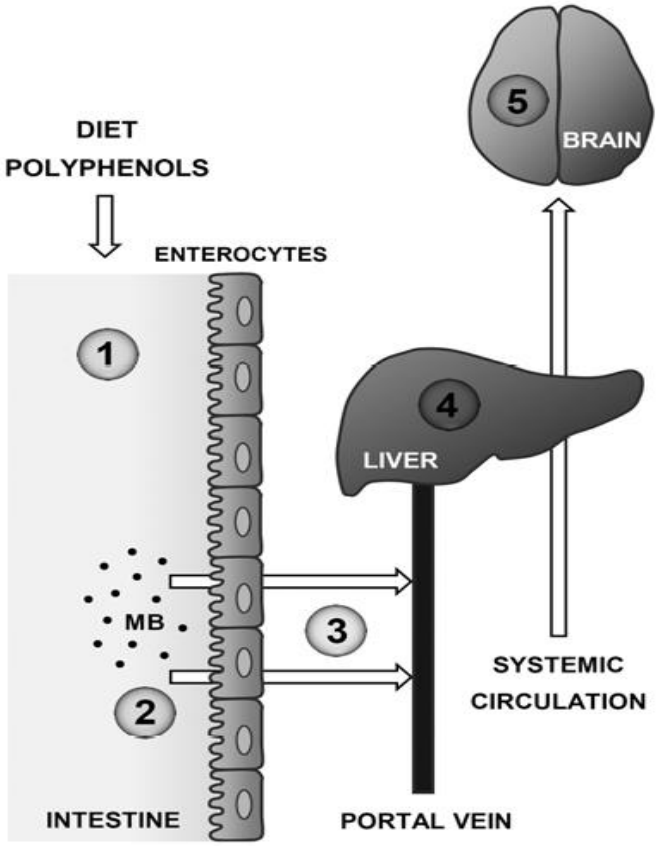


Figura 5- Representação esquemática dos principais processos que regem a biodisponibilidade de polifenóis em mamíferos. Como xenobióticos, eles são submetidos a desintoxicação para aumentar a hidrofilicidade. Etapas: 1) polifenóis são dissolvidos em fluidos intestinais e conjugado no enterócito; 2) uma fração variável é metabolizada por enzimas da microbiota do intestino humano; 3) polifenóis ou os seus metabólitos são absorvidos e dissolvidos no sangue portal; 4) uma fração é metabolizada por enzimas hepáticas; 5) a fração restante fica disponível para distribuição aos tecidos por circulação sistêmica incluindo tecido cerebral através barreira hematoencefálica (ANDRADE E ASSUNÇÃO, 2012).

Publicações recentes têm enfatizado as atividades biológicas de compostos bioativos presentes nos alimentos no corpo humano, especialmente no cérebro, no entanto muitos mecanismos são atualmente desconhecidas. O desafio é, portanto, prosseguir cautelosamente até que rigorosos ensaios clínicos controlados randomizados possam ser realizados para

determinar se polifenóis e/ou seus metabólitos têm real eficácia em indivíduos afetados por demência e outras condições neurodegenerativas (SPENCER et al., 2012; SHAFFER e HALLIWELL, 2012;VAUZOUR, 2017).

2.3 Erva-mate e eventos genotóxicos

Apesar da capacidade antioxidante e dos benefícios à saúde, alguns autores sugerem que extratos de erva-mate (*Ilex paraguariensis*) podem apresentar atividades genotóxicas e mutagênicas, contribuindo para a incidência de alguns tipos de câncer, especialmente de orofaringe e esôfago humano (WNUK et al., 2009; FONSECA et al., 2000).

De acordo com Lubin et al. (2014), beber mate aumenta o risco de carcinoma de células escamosas esofágicas, o estudo mostrou que a associação não foi influenciada pela intensidade do consumo, mas sim por altas temperaturas da água. A IARC (2016), em um encontro que discutiu a carcinogenicidade do consumo de café, mate e bebidas muito quentes classificou mate (com temperatura acima de 65^0C) como “provavelmente cancerígeno para humanos”. Uma meta-análise publicada por Chen et al. (2015), mostrou que o consumo de bebidas e alimentos quentes está associado a um aumento significativo no risco de carcinoma esofágico, especialmente nas populações asiáticas e sul-americanas, apontando que a infusão de mate pode ser um dos indutores de mutagênese.

Além da temperatura da água, o mecanismo cancerígeno pode ser a exposição a hidrocarbonetos aromáticos policíclicos (HAP), que são contaminantes originários do processo de industrialização da erva (IARC, 1991). Esses compostos são produzidos pelo tratamento térmico pelo qual passam as folhas e galhos da planta, em um processo que inclui a exposição direta ao fogo (*zapecado*), seguido da secagem onde a matéria-prima entra em contato com o ar quente e os gases de combustão provenientes da queima de madeira (HECK e MEJIA, 2007). Apesar de alguns HAPs serem genotóxicos e mutagênicos, há poucos trabalhos demostrando sua associação com o consumo de bebidas a base de erva-mate. Além disso, as ervas argentinas foram estudadas e o nível de benzo[a]pireno, o HAP mais prejudicial, não excedeu o nível máximo sugerido pela Organização Mundial da Saúde para a água potável (700 ng/L) (THEA et al.,2016).

Mutação está diretamente relacionada a reparo de DNA e um dos desfechos genotóxicos, nesses casos, é o aumento do risco de câncer, com o envelhecimento quando as mutações se acumulam como resultado de um reparo errôneo de DNA (VIJG et al., 2013; EDIFIZI e SCHUMACHER, 2015). No entanto, a recombinação mitótica tem sido descrita

como um fator preponderante no processo carcinogênico, sendo este o mecanismo que mais contribui para perda da heterozigose (RIBEIRO, SALVADORI e MARQUES, 2003).

Estudos apontam que compostos fenólicos presentes em alimentos e plantas podem exercer efeito duplo sobre o estado redox celular. Normalmente eles parecem atuar como antioxidante prevenindo carcinogênese, enquanto nas células cancerígenas seu efeito está relacionado ação pró-oxidante, promovendo apoptose (LEÓN-GONZÁLEZ et al., 2015). A ação quimiopreventiva dos polifenóis envolve sua capacidade de evitar ou reduzir os danos celulares mediados pelo estresse oxidativo, por meio da eliminação do ROS, quelação de metais ou expressão de genes que codificam enzimas desintoxicantes, entre outros mecanismos (DAI e MUMPER, 2010).

Por outro lado, os polifenóis podem afetar a atividade de várias enzimas e receptores, através da modulação do nível de expressão das proteínas ou da ligação direta em centros catalíticos (KOROBKOVA, 2015). Desta forma, eles podem alterar a atividade do citocromo P450 afetando o metabolismo e a eliminação de drogas (KYSELOVA, 2011).

As enzimas do citocromo P450 humano catalisam reações oxidativas de um amplo espectro de substratos e desempenham um papel crítico no metabolismo dos xenobióticos, como drogas e compostos dietéticos. A atividade do CYP está associada a vários fatores, incluindo o potencial redox, a conformação proteica, a acessibilidade do site ativo por substratos e outros. Considera-se que a CYP3A4 em humanos e sua homóloga CYP6g1 em *Drosophila* sejam as principais enzimas envolvidas no metabolismo de drogas e na maioria dos outros xenobióticos (RAND, LOWE e MAHAPATRA, 2012; BASHEER e KEREM, 2015).

Estudos que avaliaram a ação de compostos naturais demostraram que a ingestão regular de chá de mate, além de atenuar a ocorrência de danos induzidos por H_2O_2 pode aumentar a capacidade de reparação do DNA. A proteção é atribuída à atividade antioxidante dos compostos bioativos presentes nos extratos (BRACESCO et al., 2003; MIRANDA et al., 2008). Animais tratados com chá mate tiveram significativa redução do dano do DNA causado pela radiação UV (BARG et al, 2014). Amigo-Benavent et al. (2017) apontam que em doses fisiológicas, extratos de erva-mate reduzem a produção de ROS basal e que seus compostos fenólicos e metabólitos podem diminuir a viabilidade e a proliferação de células cancerosas, mais do que extratos de grão de café verde.

2.3.1 Teste de Mutação e Recombinação Somáticas (SMART)

O SMART da asa de *Drosophila melanogaster* baseia-se na identificação de pelos com fenótipos mutantes que representam a expressão fenotípica de lesões em nível de DNA. Tais alterações são induzidas nas células dos discos imaginais que originam as asas dos adultos com seus pelos ou tricomas. Os pelos mutantes apresentam-se como manchas, com fenótipos característicos, que indicam a ocorrência de eventos genéticos relacionados com mutações pontuais, aberrações cromossômicas e rearranjos estruturais devidos à recombinação mitótica (ANDRADE, REGULY e LEHMANN, 2004).

A análise das lesões induzidas é feita pela observação de grupos de células (clones mutantes) que expressam fenotipicamente os genes marcadores *flr*^{3} *ou mwh*, responsáveis por mudanças na forma dos pelos (GRAF et al., 1984). O tipo de mancha mutante observada nas asas dos adultos trans-heterozigotos permite a caracterização de eventos genotóxicos distintos. Manchas simples podem originar-se tanto por eventos mutacionais, incluindo mutações pontuais e aberrações cromossômicas, como também por conversão ou recombinação mitótica. Manchas gêmeas são produtos exclusivos de eventos recombinacionais (GRAF et al., 1989). A **figuras 6** apresenta o formato de uma asa de *Drosophila melanogaster* e os tipos de pelos que podem ser observados microscopicamente na sua análise.

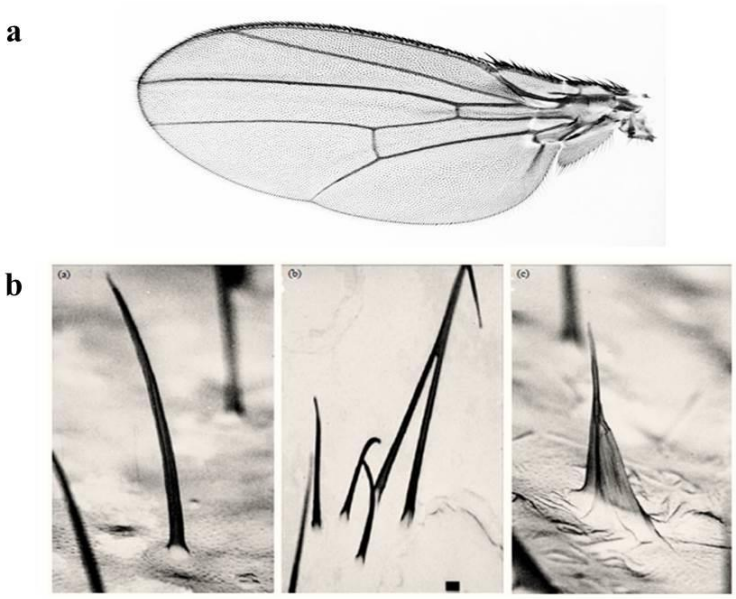


Figura 6- Asa de *Drosophila melanogaster*. (a) Microscopia da asa inteira (David Houle/Florida State University, http://www.nina.no.Consultado em 15/03/2017); **(b)** Microscopia eletrônica de mutações presentes na asa de *D. melanogaster*, (a): selvagem, (b) mutação tipo *mwh* e (c): mutação tipo Flare (*flr*^{3}) (GRAF et al. 1984, 1989).

Além disso, o teste permite a detecção de genotoxinas de ação direta e de compostos que exercem sua ação genotóxica quando metabolizados. O diagnóstico é baseado na análise de indivíduos provenientes de cruzamento padrão (ST) que utiliza linhagens com níveis basais de enzimas de metabolização (Citocromo CYP6A2) o do cruzamento aprimorado (HB) que utiliza linhagens com alto nível destas enzimas (FROLICH e WURGLER, 1989; SANER et al., 1996).

2.3.2 SMART para detecção da ação de compostos bioativos em eventos genotóxicos

SMART aplicado para investigar a capacidade de compostos biologicamente ativos atuarem como agentes antimutagênicos e/ou para avaliar a potencialidade desses compostos produzirem mutações, tem demostrado ser uma ferramenta rápida e de baixo custo.

Extratos de *Dioscorea pentaphylla*, o popular cará, co-incubados com agentes mutagênicos, levaram a reduções significativas na frequência de mutações induzidas, sendo o potencial antioxidante do extrato proposto como um dos principais mecanismos associados ao desfecho observado (PRAKASH et al., 2014). O néctar de caju mostrou capacidade de modular eventos que precedem danos induzidos e também processos de reparo do DNA (SILVA et al., 2016).

O mesmo teste, considerando o risco de danos genéticos, comprovou segurança no consumo de baixas quantidades de própolis brasileiro e confirmou a ausência de ações mutagênicas e de recombinação a partir do uso da própolis verde e marrom (RODRIGUES et al., 2016). Artepillin C, um flavonoide isolado da própolis verde demostrou capacidade de proteger contra a genotoxicidade provocada pelo indutor mitomicina-C. Essa modulação foi associada à redução do dano genético causado por eventos recombinantes, em uma condição dose-dependente (RODRIGUES et al., 2017).

Nessa linha estudos buscam, cada vez mais, aprofundar o conhecimento e a segurança no uso de compostos naturais para fins terapêuticos ou não, no entanto a aplicação de extratos de erva-mate para avaliar indução de dano do DNA, detecção de eventos recombinantes e/ou ativação de mecanismos de reparo é inédita.

3 OBJETIVOS

3.1 Objetivo geral

Analisar os efeitos dos extratos de erva-mate (*Ilex paraguariensis* A.St. Hill.) sobre marcadores de estresse oxidativo e sua relação com anti e co-genotoxicidade.

3.2 Objetivos específicos

-Avaliar a capacidade dos extratos de erva-mate modularem o equilíbrio redox e influenciarem o tempo de vida de *Drosophila melanogaster* alimentadas com uma dieta hipercolesterolêmica;

-Detectar a presença de matesaponinas em extratos de erva-mate obtidos por meio de sucessivas extrações;

-Mensurar os efeitos dos extratos de erva-mate e do CGA sobre os níveis de peroxidação lipídica e carbonilação de proteínas em regiões cerebrais de ratos submetidos à restrição de movimentos;

- Observar os efeitos dos extratos de erva-mate e do CGA sobre o balanço redox GSH/GSSG em nível de SNC;

-Observar o potencial mutagênico de extratos de erva-mate por meio Teste de Mutação e Recombinação Somática (SMART) em asas de *Drosophila melanogaster*;

-Avaliar os efeitos de extratos de erva-mate sobre danos induzidos por EMS e MMC, através do sistema de pré e pós-tratamentos, utilizando o SMART.

4 RESULTADOS

Os resultados aqui apresentados estão sob a forma de dois artigos e um manuscrito científico. O mapa conceitual abaixo exibe informações a respeito destas produções (**Figura 7**).

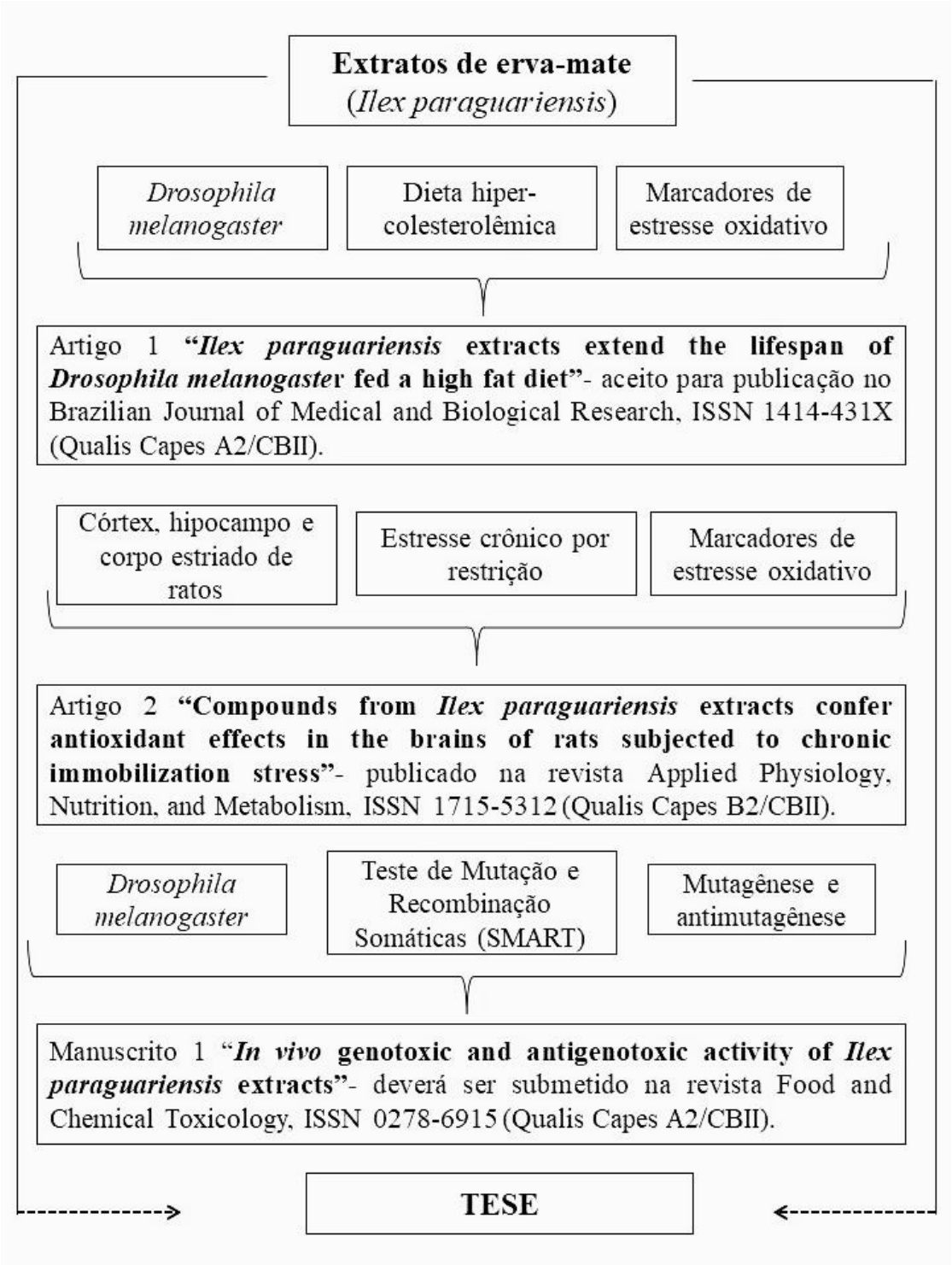


Figura 7- Mapa conceitual descrevendo as produções científicas desta tese.

4.1 Artigo 1

Brazilian Journal of Medical and Biological Research

Brazilian Journal of medical and biological research

Ilex paraguariensis extracts extend the lifespan of Drosophila melanogaster fed a high fat diet

Journal:	*Brazilian Journal of Medical and Biological Research*
Manuscript ID	6784.R1
Manuscript Type:	Full Paper
Date Submitted by the Author:	12-Jul-2017
Complete List of Authors:	Colpo, Ana; Universidade Federal do Pampa - Campus Uruguaiana Lima, Maria ; Universidade Federal do Pampa - Campus Uruguaiana da Rosa, Hemerson; Universidade Federal do Pampa - Campus Uruguaiana LEAL, ANA; UNIVERSIDADE DA REGIÃO DA CAMPANHA COLARES, CHAIANE; UNIVERSIDADE DA REGIÃO DA CAMPANHA ZAGO, ANA; UNIVERSIDADE DA REGIÃO DA CAMPANHA Salgueiro, Andréia; Universidade Federal do Pampa - Campus Uruguaiana, BERTELLI, PABLO; Universidade de Caxias do Sul MINETTO, LUCIANE; Universidade de Caxias do Sul MOURA, SIDNEI; Universidade de Caxias do Sul MENDEZ, ANDREAS; Universidade Federal do Rio Grande do Sul Folmer, Vanderlei; Universidade Federal do Pampa,
Keywords:	Yerba mate, Matesaponin, LC-MS, Antioxidants compounds, Chronic diseases, Oxidative stress
Special Sections:	Biomedical Sciences/Biochemistry and Molecular Biology

Abstract

Studies have suggested that total energy intake and diet composition affect lifespan and ageing. A high-fat diet induces oxidative stress and affects the development of diseases. In contrast, antioxidants are capable of reducing its harmful effects. *Yerba mate* beverages are an important source of antioxidants, but there is scarce knowledge about their effects on suppressing fat accumulation. Here, we investigated the compounds present in *yerba mate* extracts and assessed their effects on *Drosophila melanogaster* given a high cholesterol diet. LS-ESI-MS analysis showed the presence of matesaponins, phenolic compounds and methylxanthines in all of the examined extracts. In *Drosophila*, under extract treatment conditions, the mean lifespan was significantly extended from 38 to 43 days, there was an increase in the ability to support induced stress and decrease lipid peroxidation products. Moreover, *yerba mate* extracts recovered the glutathione S-transferases (GST) activity and reduced the cholesterol level. Taken together, our results support that extracts can extend lifespan by reducing the detrimental effect of a high fat diet in *Drosophila melanogaster*, and this outcome can be associated with the compound content in the extracts. This study improves the understanding of natural interventions that reduces stress-induced oxidative damage, which is fundamental to promoting healthy ageing.

Keywords: *Yerba mate;* Matesaponin; LC-MS; Antioxidants compounds; Chronic diseases; Oxidative stress

1 Introduction

Diets should ideally have a nutrient balance. This is an important determinant of fitness and lifespan in living organisms. Both caloric restriction and natural compounds in the diet extend the lifespan and delay the occurrence of age-related diseases in various aging models (1).

In contrast, the level of reactive oxygen species (ROS) produced by mitochondrial metabolism increases during excessive food intake (2), this reduces the lifespan and increases the risk of disease (3). A high fat diet can induce obesity, and adipose tissue generates reactive species (RS) (4,5).

In this setting, compounds with antioxidant properties can alleviate the deterioration caused by ROS and delay the aging process (5). Polyphenols may play protective roles through the hydrogen atom transfer, single electron transfer and metal chelation (6). Moreover, Ríos-Hoyo et al. (7) observed that polyphenols can evoke beneficial effects by exerting antioxidant activity and acting through metabolic pathways that enhance cardiovascular health, promoting vasodilatory, anti-atherogenic, antithrombotic, and anti-inflammatory effects. Saponins, in turn, induce reduction of the fat weight, plasma triglyceride level, and appetite as well as inhibit pancreatic lipase (8).

Ilex paraguariensis extracts are an important source of polyphenols, methylxanthines (9) and saponins (8). Many studies describe their effect on protecting against ROS and stimulating cells' antioxidant defences (9,10). Traditionally, *yerba mate* beverages are consumed in Brazil, Argentina, Uruguay, and Paraguay, named *"Chimarrão", "Mate" or "Tererê"* (11). Mate consumption occurs with sequential extractions. However, few groups have been working on analyzing the extracts obtained with this approach.

Drosophila melanogaster is considered a good pragmatic model for evaluating metabolic disorders. Fruit flies have many of the same basic metabolic functions as mammals,

including the ability to maintain glucose homeostasis, store and mobilize energy stores, and modulate food intake, and many molecular mechanisms that regulate these metabolic processes are conserved in this model (12).

In this study, using extracts obtained from *yerba mate*, we evaluated the presence of different compounds with antioxidant potential. We also explored the capacity of extracts to reduce fat accumulation, improving the stress resistance and extending the lifespan of fruit flies that were fed a high cholesterol diet.

2 Materials and methods

2.1 Extract preparation

Aqueous extracts were obtained by recreating the traditional mate preparation process. We used *yerba* Baldo®, a brand marketed in Uruguay. Mate was prepared in a medium-size gourd; the *yerba mate* level occupied two thirds of the volume in the bowl (85 g). The free volume was filled with water (70 mL) at 80^{o}C. The water was remained in contact with the *yerba mate* for one minute, and then was removed through a pump attached to a suction system. The sequential infusion (mate) extracts 1, 2, 5, 10, and 15 were stored for further analysis, all other extractions were discarded. After the extraction, mate extracts were filtered using filter paper [thickness 205 µm (J.Prolab®, S.J.dosPinhais, Brazil)], stored in microtubes and kept frozen in a freezer (-18^{o}C) until further use (9).

2.2 Liquid chromatography-electrospray ionization/multi-stage mass spectrometry (LC-ESI-MS)

LC-ESI-MS was used to confirm the presence of matesaponins, polyphenols and methylxanthine in first, second, fifth, tenth and fifteenth mates (extracts), respectively. LC analyses were conducted using a UHPLC Shimadzu device (Kyoto, Japan) equipped with a CBM-20A controller, LC-20AD pump and SIL 20AHT auto sampler. A Zorbax XDB-C8

column (150 x 4.6 mm, 5 µm) was used. The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) at a flow rate of 0.3 mL/min according to the following gradient: 0.01-1 min, 90% solvent A; 1.01-4 min, 90-65% solvent A; 4.01-7 min, 65% solvent A; 7.01-11 min, 65-50% solvent A; 11.01-14 min, 50% solvent A; 14.01-17 min, 50-10% solvent A; 17.01-21 min, 10% solvent A; 21.01-23 min, 10-90% solvent A; and 23.01-30 min, 90% solvent A. The injection volume was 10 µL and the analysis was performed at 20^{o}C. The mobile phase was prepared daily, filtered through a 0.45-µm membrane filter (Millipore) and sonicated before use. The MS analyses were performed on a micrOTOF-QII (Bruker® Scientific; Billerica, MA, USA) with an electrospray ionization interface (ESI). TOF control data acquisition software was used. LC/ESI/MS was conducted in the positive-ion mode and operated under the following conditions: nitrogen gas temperature of 200^{o}C, drying gas flow rate of 8 L/min, capillary voltage of 4000 eV, and ionization energy of 3 eV. Mass spectra were recorded in the full scan mode in the m/z range 50-1400.

2.3 Fly strains and culture conditions

Wild type *Drosophila melanogaster* - Harwich strains were obtained from the National Species Stock Center (Bowling Green, OH, USA). Flies were grown in the Clinical Analysis Laboratory (LEAC) from Universidade da Região da Campanha (URCAMP), where they were maintained on the cornmeal medium at 25^{o}C with a 12-hr light/dark cycle.

2.4 Diets

The standard diet (SD) used to keep the stocks available was prepared according of previously described formulation by Bahadorani and Hilliker, with modifications (13). Briefly, 1 liter of the standard diet consisted of 750 mL of water, 37.5 g of dry yeast, 7.5 g of agar, 138 g of corn flour, 65 g of crystal sugar, 0.0038 g of nipazol and 3.5 mL of acid solution (10 mL of phosphoric acid and 100 mL of acetic acid) into water in a sealed

chamber. In order to obtain flies grown from eggs exposed to a high cholesterol diet, 1% of synthetic cholesterol (3β-Hydroxy-5-cholestene,5-Cholesten-3β-ol, Sigma Aldrich) was added to the standard diet. The diet enriched with synthetic cholesterol was named the experimental diet (ED). To generate the stocks, 80 mL of the cooked mixture was poured into each vial.

For the fat-induced damage experiments the synthetic cholesterol was added into the SD at 1% on a weight basis. Because synthetic cholesterol is insoluble in water it was dissolved in Dimethyl sulfoxide (DMSO). Synthetic cholesterol was added to 100 mL of DMSO (10 mM) and 5 mL of this solution was added to 1000 g of food.

2.5 Exposure to *yerba mate* extracts

Male flies (2-3 days old) were divided into different groups and were reared in vials; 30 mL of the cooked mixture was poured per vial. In this study, only male flies were used because there is less hormonal effect in the male than in the female flies. All experimental protocols were performed in triplicate and had two control groups; one with the SD was named the control standard diet (CSD) and another one with the ED was named the control experimental diet (CED). In the ED+ mates, the 1st, 2nd, 5th, 10th, and 15th mate extract fractions (1 mL) were added into the warm food, and they were mixed with a small spatula. In the control groups, we used water instead of mate. The number of flies varied according to the protocols, as shown in **Figure 1.**

2.6 Lifespan and body weight

To evaluate the effects of *yerba mate* extracts on the fly lifespan and body weight, we used a protocol that was previously described by Peng et al. with minor modifications (14). Each group of 20 male flies was exposed to ED with the 1st, 2nd, 5th, 10th, and 15th mates (1 mL of each extract). The controls (CED and CSD) received water in the same volume. Dead flies were counted every 2-3 days, and the remaining flies were transferred to a new vial containing the same diet. Feeding lasted for 43 days.

The change in the average body weight per fly was used as an indicator of whether *yerba mate* extracts affect the food intake of the fruit flies. *D. melanogaster* in each vial were anesthetized with Ether (Diethyl ether ACS reagent) and, on the same days in which diet vials were changed, they were weighed in an analytical balance (BEL®). The same insects that were subjected to the lifespan protocol were part of this assay. The average body weight per fly in each group was recorded. To confirm food consumption, we used the dye method in accordance with the protocol described by Verspoor et al. (15).

2.7 Stress tolerance

2.7.1 Paraquat and hydrogen peroxide (H_2O_2) treatments

To examine the resistance of flies to stress induced by Paraquat (1,10-dimethyl-4,40-bi-pyridinium dichloride; Pq^{2+}) or H_2O_2, we used the protocols proposed by Peng et al. with minor modifications (14). Flies (n = 20 per vials) were maintained on either the standard diet or on experimental diet containing mate extracts. All flies were raised in 25^{o}C. On day 10, fruit flies in the groups were first starved for 2 h and then transferred to new vials containing a filter paper saturated with 1 mL of 20 Mm paraquat that was diluted in a 6% glucose solution or 1 mL of 30% H_2O_2 diluted in 6% glucose. The number of dead flies was counted every 4-6 h until all flies were dead.

2.7.2 Cold and starvation resistance tests

The cold and starvation resistance tests were based on the method previously described by Heinrichsen and Haddad (16). In short, for cold resistance, a -5^{o}C bath was made using water, ice and salt. Each group of 20 flies was placed in empty plastic vials and into the water bath. They remained as such for 2 hours while the temperature was regularly checked throughout. At the end of the 2-hour period, vials were removed from the water bath and flies were transferred to regular food and left to recover at room temperature. After 24 hours, survival was recorded as the number of flies that had regained consciousness.

To analyze starvation, each group of 20 flies was placed in a plastic vial without food. A small, circular filter paper was placed in the bottom of the vial with 75 µL of water to prevent dehydration, and the water was replenished with every 16 hours or as needed. Survival was recorded every 4-8 hours according to the number of flies alive in each vial.

2.8 Metabolic parameters and enzymatic activity

The metabolic determinations and enzymatic activity were evaluated from homogenized pools of animals. Pools of 10 flies were used to measure the enzymatic activity, 20 flies to evaluate triglycerides, and 50 flies to evaluate the total lipid extraction and cholesterol quantification. All determinations were performed three times with groups treated in the same conditions.

In these experiments, immediately after removing the flies from the treatment vials, they were frozen in liquid nitrogen and then rinsed with 1 mL of cold PBS to remove all traces of food that might be attached to the animal body (17).

2.8.1 Cholesterol

To quantify the cholesterol levels, we extracted the total lipids from fly samples. For this purpose, we used a pool of 50 flies that were homogenized in 1250 µL of 50 mM potassium phosphate buffer (TFK). The homogenate underwent extraction in a chloroform-methanol-water solution according to the lipid extraction method described by Bligh and Dyer (18).

Cholesterol was measured using the Cholesterol-Liquiform Labtest kit®. This kit uses cholesterol oxidase to convert free cholesterol to cholest-4-en-one and hydrogen peroxide. Phenol and 4-aminoantipyrine are oxidized yielding quinoneimine, which has the maximum absorbance at 500 nm. The results are expressed as mg/dL of tissue.

2.8.2 Triglycerides

The homogenate was centrifuged at 3000x*g* for 10 min and the supernatant was then aliquoted. Triglycerides were measured according to the reactions of lipase, glycerokinase, 1-P-glycerol oxidase, and peroxidase enzymes using the Triglycerides Liquiform-Labtest kit®. The results are expressed as mg/dL of tissue.

Since Tennessen et al. (17) observed that the presence of eye pigment in adult samples could interfere with accurate absorbance measurements at certain wavelengths, we tested the assay, before the analysis, at 505 nm using flies with or without heads and observed that the eye pigment did not have an effect at this wavelength.

2.8.3 Glutathione S-transferase (GST)

The glutathione S-transferase (GST) activity was measured as described by Habig and Jakoby (19) using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. One unit of GST was calculated as µmol of CDNB conjugate per mg protein ($U.mg^{-1}$ Protein), using the molar extinction coefficient of 9.6 mM^{-1} cm^{-1}. The enzymatic activity was expressed as GST units/dL protein.

2.9 Thiobarbituric acid reactive species (TBA-RS) levels

Lipid peroxidation was assayed according to a method proposed by Ohkawa et al. (20). Briefly, fly homogenate (1:20 w/v) was mixed in a medium containing 8.1% sodium dodecyl sulfate, acetic acid buffer (pH 3.5), and 0.8% aqueous solution of thiobarbituric acid. After heating at 95^{o}C for 120 min, the red pigment produced was spectrophotometrically measured at 532 nm. The results were calculated using a standard curve of malondialdehyde (MDA) and corrected by tissue milligrams. The results are expressed as nanomoles of MDA per milligram of tissue.

2.10 Protein carbonyl levels

Protein carbonyl was measured according Levine et al. (21) with some modifications. Fly homogenates were derivatized using 2,4-dinitrophenylhydrazine (DNPH). DNPH reaction

proteins were precipitated with an equal volume of 20% trichloroacetic acid and, after centrifugation (12.000 rpm, 15 min, 4^{o}C), they were washed three times with an ethanol/ethyl acetate mixture (1:1). Finally, the precipitates were dissolved in 2% sodium dodecyl sulfate. Protein carbonyl levels were spectrophotometrically determined at 370 nm, compared to blanks. The results were calculated using the molar extinction coefficient of DNPH, which was corrected by the protein content and expressed as nanomoles of carbonyl per milligram of protein.

2.11 Statistical analysis

Data are expressed as the means ± standard deviations. For survival and longevity analysis, we performed a dose-response curve and plotted the sigmoidal dose response curve using nonlinear regression followed by the Shapiro-Wilk and Logrank tests. All other data were subjected to one-way ANOVA followed by the Dunnett's test. Differences between groups were considered significant at $p \leq 0.05$.

3 Results

The choice of herb used in this study was based on a previous report from our group. The Uruguayan brand Baldo® presented the higher content of polyphenols and methylxanthines. Moreover, compared with herbs from Argentina and Brazil, this brand has the most effective antioxidant capacity (9,10).

Moreover, in the quoted study was observed that all compounds examined showed a similar decrease in concentration over subsequent extractions. The concentrations of the compounds analysed, by HPLC analysis, was quantified as follows: chlorogenic acid > caffeic acid > caffeine > theobromine (9).

3.1 LC-ESI-MS

Aqueous *Ilex paraguariensis* extract, prepared as mate or chimarrão beverage, was monitored by HPLC-DAD and submitted to ESI-MS analyses. Standards, UV spectra, MS+ fragmentation and previous reports confirmed the presence of matesaponins, phenolic compounds and methylxanthines in all samples. **Table 1** lists the identified compounds, their retention times (Rt), molecular ions [M+H]+ and fragment ions, whereas **figures 2** and **3** illustrate the mass spectra. It is remarkable that all the nine components identified were detected during the successive extractions, being present in all mate tested.

3.2 Lifespan and body weight

Herein, fruit flies exposed to a high cholesterol diet were treated with *yerba mate* extracts. The corresponding analyses revealed that mate treatment promotes increased lifespan. The maximum lifespans for the mate 1 and 2 treated groups were 42 and 43 days, respectively, compared with 38 days for flies on an experimental diet and 40 days for flies on the standard diet. There was no statistically significant difference between the controls and other extracts (mates). The **Figure 4** presents the longevity curve in the groups where there was statistical significance.

No significant differences in body weight were observed between the two controls and *yerba mate* extract-treated groups. The diet consumption in all experimental groups has been confirmed by the intestinal flies' coloration (blue dye, **Figure 1 in supplementary material)**.

3.3 Stress tolerance

To investigate whether the use of antioxidant compounds in high cholesterol diet conditions can affect the ability of flies to resist stress, we used four protocols. The first two examined the resistance to superoxide and OH^- induced stress, whereas the other two verified cold and starvation tolerance in *Drosophila melanogaster*.

The results from the paraquat challenge test showed that flies exposed to the CED had less resistance ($p<0.01$) than those exposed to the CSD. When the groups treated with mates

were compared to the CED, we confirmed that the resistance was significantly improved in flies receiving mates 1, 2 and 5 ($p<0.01$). The maximum survival time was 120 h in flies exposed to the CSD, and for the groups treated with mates 1 and 2. The minimum survival time was 96 h for the CED and mate 15-treated group. **Figure 5A** depicts these results.

In addition, flies were exposed to H_2O_2 to examine their resistance to OH^- induced oxidative stress. The insects were also susceptible to damage produced by this condition. The CED fly groups had a lower capacity to resist to this free radical species when they were compared to the CSD group and ED+mate-treated group ($p<0.05$). There were no significant differences between the ED+ mate and CSD **(Figure 5B).**

With respect to the capacity to resist starvation conditions, flies consuming diets with cholesterol supplementation were more resistant to starvation than flies receiving standard diet. After 48 hours of starvation, 80% of flies in the CSD died, in contrast to 55% of flies in the CED and 35% of flies in the ED+mates (**Figure 5C**). The starvation resistance was significantly higher in flies fed CED and ED+mates compared to the CSD ($p<0.05$). To complete, the data showed that flies in ED did not present with total recovery after two hours to cold exposure (**Figure 5D)**.

3.4 Metabolic parameters

To quantify the flies' cholesterol levels, we extracted the total lipids from samples. Our results showed that there were changes in the cholesterol levels in flies with different treatments (**Figure 6A**). Comparing the cholesterol levels in the CSD (217.4±15.8) and CED (291.9±7.8), we found a significant increase in the cholesterol levels in flies that were fed with an experimental diet ($p<0.05$). Flies given ED+mate (all groups tested) presented with lower cholesterol levels than those given ED alone ($p<0.01$). In terms of triglyceride (TAG), no significant differences were observed in the tested treatments (**Figures 6B**).

3.5 GST activity

GST activity was reduced in a pool of flies in the CED compared to in the CSD and ED+mates. It was possible to observe that the *yerba mate* extracts increased their activity in a significant manner ($p<0.01$; **Figure 7**).

3.6 Thiobarbituric acid reactive species (TBA-RS) and protein carbonyl levels

Lipid peroxidation and protein carbonylation are induced by a high cholesterol diet, which can be reduced by the *yerba mate* extracts. Our data showed an increase in the TBA-RS and protein carbonylation level in the CED group compared to the CSD ($p<0.05$). In these two experiments, mates effectively reduced the malondialdehyde and carbonyl levels (reactive intermediates from lipid peroxidation and protein carbonylation) at standard diet (CSD) level (**Figures 8 A and B**). Results showed that mates 5, 10 and 15 were more effective to reduce the TBA-RS and protein carbonylation levels.

4 Discussion

The research describes an investigation about the effect of *yerba mate* on the lifespan of *Drosophila melanogaster*. First, we evaluated the extracts to confirm the presence of the target compounds. A previous study by our group has described some phenolic compounds and methyxanthines from *yerba mate* (10). Here, we also detected five metasaponis in the extract. According to the literature, metasaponins in *Ilex paraguariensis* samples mainly consist of ursolic acid derivatives (22).

Matesaponin 1, which had a molecular ion *m/z* of 913.51 $[M+H]^+$ with two losses of 162 Da and one loss of 150 Da, corresponding to two glucosyl and one arabinosyl residues (23). Fragmentation associated with loss of sugar molecules is common, because matesaponins are glycosylated compounds. Previous studies mainly report the presence of glucose, arabinose and rhaminose sugars linked to *Ilex paraguariensis* saponins (22, 24,25).

In sequence, the ion at m/z 1059.57 $[M+H]^+$ for matesaponin 2 gave rise to fragments that are consistent with losses of four sugar moieties, which we identified as 2Glc-Rha-Ara, resulting in *m/z* 439 (ursolic acid aglycone).

Matesaponin 3 (*m/z* 1075.56) has fragments at 913, 751 and 439, which have corresponding losses of four saccharide units (3Glc-Ara). Matesaponin 4 gave an ion at *m/z* 1221 $[M+H]^+$ with fragments at *m/z* 1059, 897, 765, 603 and 439, confirming the loss of (Glc-Rha-Ara-Glc-Glc-Glc) as a linear sequence and resulting in aglycone. The matesaponin 5 structure had a molecular ion at *m/z* 1383 with losses of six sugar moieties, which is consistent with a multiply glycosylated ursolic acid ester (22).

Positive ESI-MS also confirmed the presence of ions $[M+H]^+$, chlorogenic acid (*m/z* 353), caffeic acid (*m/z* 181), caffeine (*m/z* 195) and theobromine (*m/z* 181), as seen in **Table 1**. Although these compounds have not generated fragments, previous HPLC-DAD analysis, with standards, confirmed their presence (9).

Previously, we had reported that the *yerba mate* extracts had significant antioxidant capacity and could increase the longevity and survival of *Caenorhabditis elegans* (9,10). Our hypothesis was that these extracts contain compounds that could target multiple molecular pathways to produce protection against oxidative stress, and that this may be important in its ability to extend lifespan. Other studies also demonstrated that extracts obtained from natural foods increase the *Drosophila melanogaster* lifetime. Blueberry extracts prolong the mean lifespan of fruit flies by 10% (1); also black tea improves the survival time of fruit flies (14). Wang et al. demonstrated that cranberry anthocyanin extract extended the *D. melanogaster* lifespan and observed that this activity was directly attributable to its antioxidant activity after absorption (5).

Despite the fact that the molecular mechanism by which polyphenol compounds extend lifespan is not entirely known, it is true that high fat diet can induce higher levels of

reactive oxygen species, as evidenced by hydrogen peroxide (H_2O_2) emission from the mitochondria. This reflects that oxidative phosphorylation is more active, requiring more reducing equivalents (4). Moreover, a fat diet increases the iron absorption and affects its regulation and use (26). This is relevant because iron plays a central role generating free radicals. In fact, plant extracts can chelate Fe^{2+} and reduce its availability for interacting with H_2O_2, which decreases the hydroxyl radical formation via the Fenton reaction (27).

Additionally, there is growing evidence that phytochemicals prolong the lifespan by modulating the network signaling pathways (28). Lima et al. (10) suggested that the effect in longevity observed in *C. elegans* treated with *yerba mate* extracts was linked to a decrease in reactive oxygen species and to an increase in the DAF-16 migration into the cell nucleus, which is probably due to the interaction between molecular pathways in the presence of high polyphenols levels.

In addition, flies with higher cholesterol levels were less capable of responding to free radicals. It occurs probably because feeding dietary fats modulates changes in the lipids and can initiate a sequence of events that lead to cell damage. In this context, failing to keep H_2O_2 low promotes considerable toxicity due to the production of highly reactive species, such as hydroxyl radicals (29). *Yerba mate* extracts delayed the harmful cycle. Phenolic compounds can donate an electron to O_2**.-** (e.g.), which was accompanied by a proton-transfer process to produce a phenoxyl radical (30), the latter radical is more stable and less reactive.

It was also observed that the flies submitted ED+mates were more resistant to starvation conditions, and that flies fed in ED were unable to recover after exposure to severe cold. Once metabolic adaptations are important to survival, these responses can be associated first to the lipids stored within the fat body that are used as a vital resource to survive through periods of low nutrient availability (31). Flies given a high fat diet are unable to activate some proteins and genes, jeopardizing the cell membrane integrity and impairing the ability to

recover from cold stress (16). The role of the compounds present in extracts, in these two effects, could be related to the fat oxidation control that ensures the thermogenesis, facilitating the regulation of fat mobilization and reducing lipid peroxidation level, which would also increase the cell membrane resistance.

Hypercholesterolemia is associated with the deterioration of antioxidant status, resulting from increased MDA levels (32). In addition high fat diet causes cholesterol accumulation, it can also induce hyperglycemia and insulin resistance, two alterations that are linked to oxidative stress. Hydrogen peroxide impairs insulin signaling and inhibits glucose transport (26). We propose that reducing the levels of cholesterol in male flies fed with an experimental diet supplemented with *yerba mate* extracts, can be attributable to an association between antioxidant effects and enzymatic/transcriptional mechanisms, which regulated and modulate DHR96 expression. The DHR96 nuclear receptor plays an essential role in coordinating the TAG, cholesterol breakdown, absorption and trafficking (33). The increase in the cholesterol levels was not sufficient to promote deposits in flies' tissues. The average weight did not vary substantially, which is in accordance with this result. This effect can also be related to the DHR96 regulation. Future experiments in flies with elevated or decreased DHR96 expression levels will help to inform us on the role of the extracts in these parameters.

The DHR96 nuclear receptor also regulates the xenobiotic responses in *Drosophila* (33). There are several possible mechanisms by which the metabolic functions of DHR96 could affect detoxification responses, both in the exchange of dietary nutrients as well as in the coordination of xenobiotic and metabolic responses within the animal (34). Excess fat consumption plays a crucial role in activating high fat diet modulated lipid metabolic pathways and can deregulate the glutathione levels (35). The results described by Curtis et al.

(36), which focus on the role of GSTA4, suggest that the GSTA4 downregulation increases the protein carbonylation and alters the glucose and lipid metabolism.

Although we have not assessed the ROS levels, increased TBA-RS and carbonyl levels can be used as an indirect evidence of high ROS production (37). Considering that the lipid peroxidation is a self-propagating autocatalytic process producing several potent ROS (3), and that the fatty acid composition of membranes might be important in aging processes, our data support the role of antioxidants in protecting lipid oxidative damage and the function of GST in the detoxification process against lipid peroxidation derived products (38).

We believe that extracts' effects are optimal even in low compounds concentrations, which agrees with the definition that antioxidant is a substance that, when present at low concentrations compared to an oxidizable compound, delay or prevent oxidative damage caused by the presence of ROS. Moreover, it is essential to consider that extracts of natural products are complex mixtures of different bioactive compounds that may act synergistically to determine its effects (39). The antioxidant intervention, based on compounds that act as free radical scavengers to detoxify oxidative-derived carbonyl reaction products, represents a new therapeutic target to trap the lipid-derived reactive carbonyl species (40).

In conclusion, *yerba mate* extracts in diet could prolong the mean lifespan, alleviate induced mortality and recover the enzymatic detoxification functions in fruit flies. The antiaging activity and antioxidant effects of *yerba mate* were associated mainly with the compounds in extracts. Despite the present observations, revealing the beneficial properties of *Ilex paraguariensis* beverages, energy metabolism in flies is likely associated with complex mechanisms in which the diet can positively or negatively affect the events involved in these interactions. Future studies should address the issue of bioavailability and metabolism of matesaponins, phenolic compounds and methylxanthines in fruit flies.

Acknowledgements

This study was supported by FAPERGS, CAPES, CNPq, UNIPAMPA and URCAMP.

References

(1) Peng C, Zuo Y, Kwan KM, Liang Y, Ma KY, Chan HYEC, et al. Blueberry extract prolongs lifespan of *Drosophila melanogaster*. *Exp. Gerontol* 2012; 47, 170-178.
(2) Choksi KB, Nuss JE, De Ford JH. Papaconstantinou J. Mitochondrial electron transport chain functions in long-lived Ames dwarf mice. *Aging* 2011; 3(8), 754-767.
(3) Halliwell, B & Gutteride, JMC. Free radicals in biology and medicine. Ed.4, New York, Oxford University Press; 2007.
(4) Li Y & Periwal V. Synergy in free radical generation is blunted by high-fat diet induced alterations in skeletal muscle mitochondrial metabolism. *Biophys. J* 2013; 104, 1127-1141.
(5) Wang L, Li YM, Lei L, Liu Y, Wang X, Ma KY, Chen ZY. Cranberry anthocyanin extract prolongs lifespan of fruit flies. *Exp. Gerontol* 2015; 69, 189-195.
(6) Leopoldini, M, Russo, N, Toscano, M. The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chem* 2011; 125(2), 288-306.
(7) Ríos-Hoyo A, Cortés MJ, Ríos-Ontiveros H, Meaney E, Ceballos G, Gutiérrez-Salmeán G. Obesity, Metabolic Syndrome, and Dietary Therapeutical Approaches with a Special Focus on Nutraceuticals (Polyphenols): A Mini-Review. *Int J Vitam Nutr Res* 2014; 84(3-4), 113-123.
(8) Resende PE, Verza SG, Kaiser S, Gomes LF, Kucharski LC, Ortega GG. The activity of mate saponins (*Ilex paraguariensis*) in intra-abdominal and epididymal fat, and glucose oxidation in male Wistar rats. *J. Ethnopharmacol* 2012; 144, 735-740.
(9) Colpo AC, Rosa H, Lima ME, Pazzini CE, de Camargo VB, Bassante FE, Puntel R, Ávila DS, Mendez A, Folmer V. Yerba mate (*Ilex paraguariensis* St. Hill.)-based beverages: How successive extraction influences the extract composition and its capacity to chelate iron and scavenge free radicals. *Food Chem* 2016; 209,185-195.
(10) Lima ME, Colpo AC, Salgueiro WG, Sardinha GE, Avila DS, Folmer V. *Ilex paraguariensis* extract increases lifespan and protects against the toxic effects caused by paraquat in *Caenorhabditis elegans*. *Int J Environ Res Public Health* 2014; 1(10), 10091-10104.
(11) Bracesco N, Sanchez AG, Contreras V, Menini T, Gugliucci A. Recent advances on *Ilex paraguariensis* research: Minireview. *J Ethnopharmacol* 2011. 136(3), 378-84.
(12) Trinh I & Boulianne GL. Modeling Obesity and Its Associated Disorders in *Drosophila*. *Physiology (Bethesda)* 2013; 28(2), 117-124.
(13) Bahadorani S, Bahadorani P, Phillips JP, Hilliker AJ. The effects of vitamin supplementation on *Drosophila* life span under normoxia and under oxidative stress. *J Gerontol A Biol Sci Med Sci* 2008;63(1), 35-42.
(14) Peng C, Chan HY, Li YM, Huang Y, Chen ZY. Black tea theaflavins extend the lifespan of fruit flies. *Exp. Gerontol* 2009; 44, 773-783.
(15) Verspoor RL, Heys C, Price TAR. Dyeing Insects for Behavioral Assays: the Mating Behavior of Anesthetized *Drosophila*. *J Vis Exp* 2015; 98, e52645.
(16) Heinrichsen ET & Haddad GG. Role of high-fat diet in stress response of *Drosophila*. ***PLoS One*** 2012; 7(8), 1-8.
(17) Tennessen JM, Barry WE, Cox J, Thummel CS. Methods for studying metabolism in *Drosophila*. ***Methods*** 2014; 15: 68(1), 105-115.
(18) Bligh EG & Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Physiol Pharmacol* 1959; 37(8), 911-917.
(19) Habig WH & Jakoby WB. Assays for differentiation of glutathione S-transferases. *Methods Enzymol* 1981; 77, 398-405.
(20) Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95(2), 351-358.

(21) Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER. Determination of carbonyl content in oxidatively modified proteins. *Methods in Enzymology* 1990; 186, 464-478.

(22) Kraemer KH, Taketa AT, Schenkel EP, Gosmann G, Guillaume D. Matesaponin 5, a highly polar saponin from *Ilex paraguariensis*. Phytochemistry 1996; 42, 1119-1122.

(23) de Souza LM, Dartora N, Scoparo CT, Cipriani TR, Gorin PA, Iacomini M, Sassaki GL. Comprehensive analysis of maté (*Ilex paraguariensis*) compounds: Development of chemical strategies for matesaponin analysis by mass spectrometry. *J. Chromatogr. A* 2011; 1218, 7307- 7315.

(24) Gnoatto SCB, Schenkel EP, Bassani, VL. HPLC method to assay total saponins in *Ilex paraguariensis* aqueous extract. *J Braz Chem Soc* 2005; 16, 723-726.

(25) Dartora N, De Souza LM, Santana- Filho AP, , 2011. UPLC-PDA–MS evaluation of bioactive compounds from leaves of *Ilex paraguariensis* with different growth conditions, treatments and ageing. *Food Chem* 2011; 129, 453-1461.

(26) Folmer V, Soares JCM, Gabriel D, Rocha JB. A High Fat Diet Inhibits-Aminolevulinate Dehydratase and Increases Lipid Peroxidation in Mice (Mus musculus). *Bioch Mol Act of Nutr* 2003; 133 (7), 2165-2170.

(27) Salgueiro ACF, Leal CQ, Bianchini MC, Prado IO, Mendez AS, Puntel R et al.The influence of *Bauhinia forticata* Link subsp. *Pruinosa* tea on lipid peroxidation and non-protein SH groups in human erythrocytes exposed to high glucose concentrations. *J Ethnopharmacol* 2013; 148(1), 81-87.

(28) Leonov A, Arlia-Ciommo A, Piano A, Svistkova V, Lutchman V, Medkour Y, Titorenko VI. Longevity Extension by Phytochemicals. *Molecul*es 2015; 20, 6544-6572.

(29) Liochev SI. Reactive oxygen species and the free radical theory of aging. *Free Radic Biol* Med 2013; 60, 1-4.

(30) Matsuoka M & Jin J. Application of Electrochemiluminescence for the Evaluation of the Antioxidant Capacity of Some Phenolic Compounds Against Superoxide Anion Radicals. *Anal Sci* 2015; 31, 629-634.

(31) Arrese EL & Soulages JL. Insect fat body: energy, metabolism, and regulation. *Annu Rev Entomol* 2010; 55, 207-225.

(32) Lassoued I, Trigui M, Ghlissi Z, Nasri R, Jamoussi K, Kessis M, et al. Evaluation of hypocholesterolemic effect and antioxidant activity of Boops boops proteins in cholesterol-fed rats. *Food Funct.* 2014; 5, 1224-1231.

(33) Sieber MH & Thummel CS. The DHR96 Nuclear Receptor Controls Triacylglycerol Homeostasis in *Drosophila. Cell Metab* 2009; 10(6), 481-490.

(34) King- Jones KK, Horner MA, Lam G, Thummel CS. The DHR96 nuclear receptor regulates xenobiotic responses in *Drosophila. Cell Metab* 2006; 4, 37-48.

(35) Ibrahim A, Natarajan S, Ghafoorunissa R. Dietary trans-fatty acids alter adipocyte plasma membrane fatty acid composition and insulin sensitivity in rats. *Metab Clin Exp* 2005; 54 (2), 240-246.

(36) Curtis JM, Grimsrud PA, Wright WS, Xu X, Foncea RE, Graham DW, et al. Downregulation of Adipose Glutathione S-Transferase A4 Leads to Increased Protein Carbonylation, Oxidative Stress, and Mitochondrial Dysfunction. *Diabetes* 2010;59,1132-1142.

(37) Salgueiro ACF, Folmer V, Silva MP, Mendez ASL, Zemolin APP, Posser T, et al. Effects of *Bauhinia forficata* Tea on Oxidative Stress and Liver Damage in Diabetic Mice. *Oxid Med Cell Longev* 2016; 1-9.

(38) Ayala A, Muñoz MF, Argüelles S. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxid Med Cell Longev* 2014; 2014, 1-31.

(39) Salgueiro ACF, Folmer V, Rosa HS, Costa MT, Boligon AA, Paula FR, et al. *In vitro* and *in silico* antioxidant and toxicological activities of *Achyrocline satureioides*. *J Ethnopharmacol*. 2016; 194,6-14.
(40) Suzuki JY, Carini M, Butterfield DA. Protein Carbonylation. *Antioxid Redox Signal* 2010; 12 (3), 323-325.

Table

Table 1- Chemical components identified in *yerba mate* extract with corresponding retention times, quasi-molecular ions in the positive mode and key fragments by LC/ESI-MS.

Compound	***Rt*** **(min)**	**Ion** **$[M+H]^+$**	**MS^+fragmentation (*m/z*)**
Matesaponin 1	15.9	913	751[M+H-Glc]; 589[M+H- 2Glc]; 439[M+H- 2Glc-Ara]
Matesaponin 2	15.5	1059	897[M+H-Glc]; 735[M+H-Glc-Rha], 603[M+H-Glc-Rha-Ara]; 439 [M+H- 2Glc-Rha-Ara]
Matesaponin 3	12.4	1075	913 [M+H-Glc]; 751[M+H-2Glc]; 439[M+H- 3Glc-Ara]
Matesaponin 4	13.0	1221	1059[M+H-Glc]; 897[M+H-Glc-Rha]; 765[M+H-Glc-Rha-Ara]; 603[M+H-2Glc-Rha-Ara]; 439[M+H- 3Glc-Rha-Ara]
Matesaponin 5	14.9	1383	1221[M+H-Glc]; 1059[M+H-Glc-Rha]; 603[M+H-2Gluc-Rha-Ara]; 439[M+H- 4Glc-Rha-Ara]
Chlorogenic acid	10.0	355	163 [M+H-195]
Caffeic acid	9.1	182	163 [M+H-H2O]
Caffeine	10.3	195	FND
Theobromine	6.6	181	FND

Figure Captions

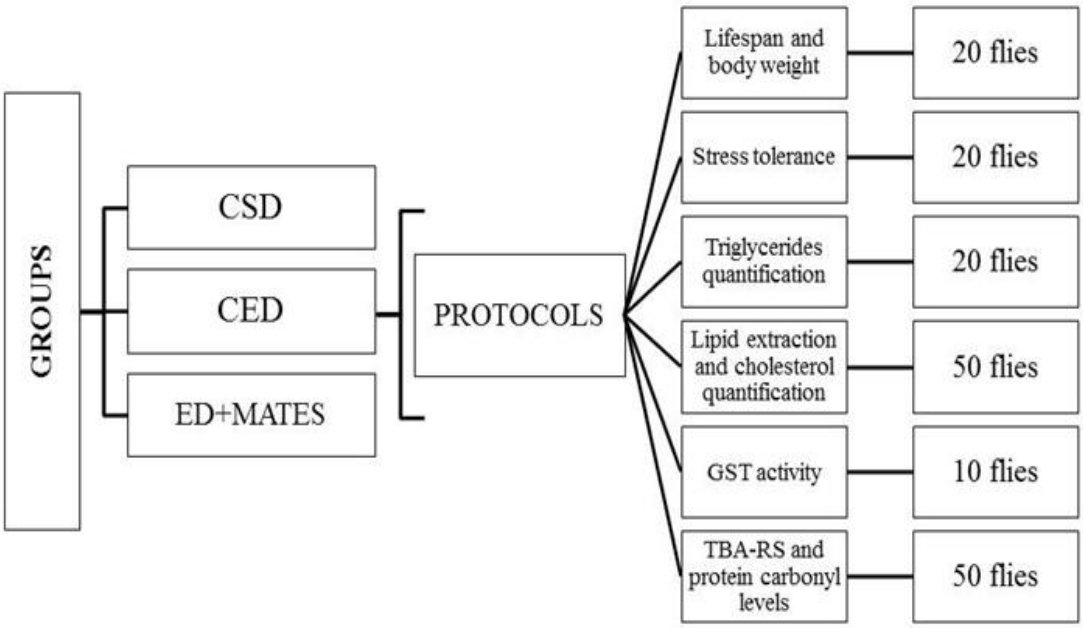


Figure 1- Flow diagram of the trial design. All experiments were performed in triplicate.

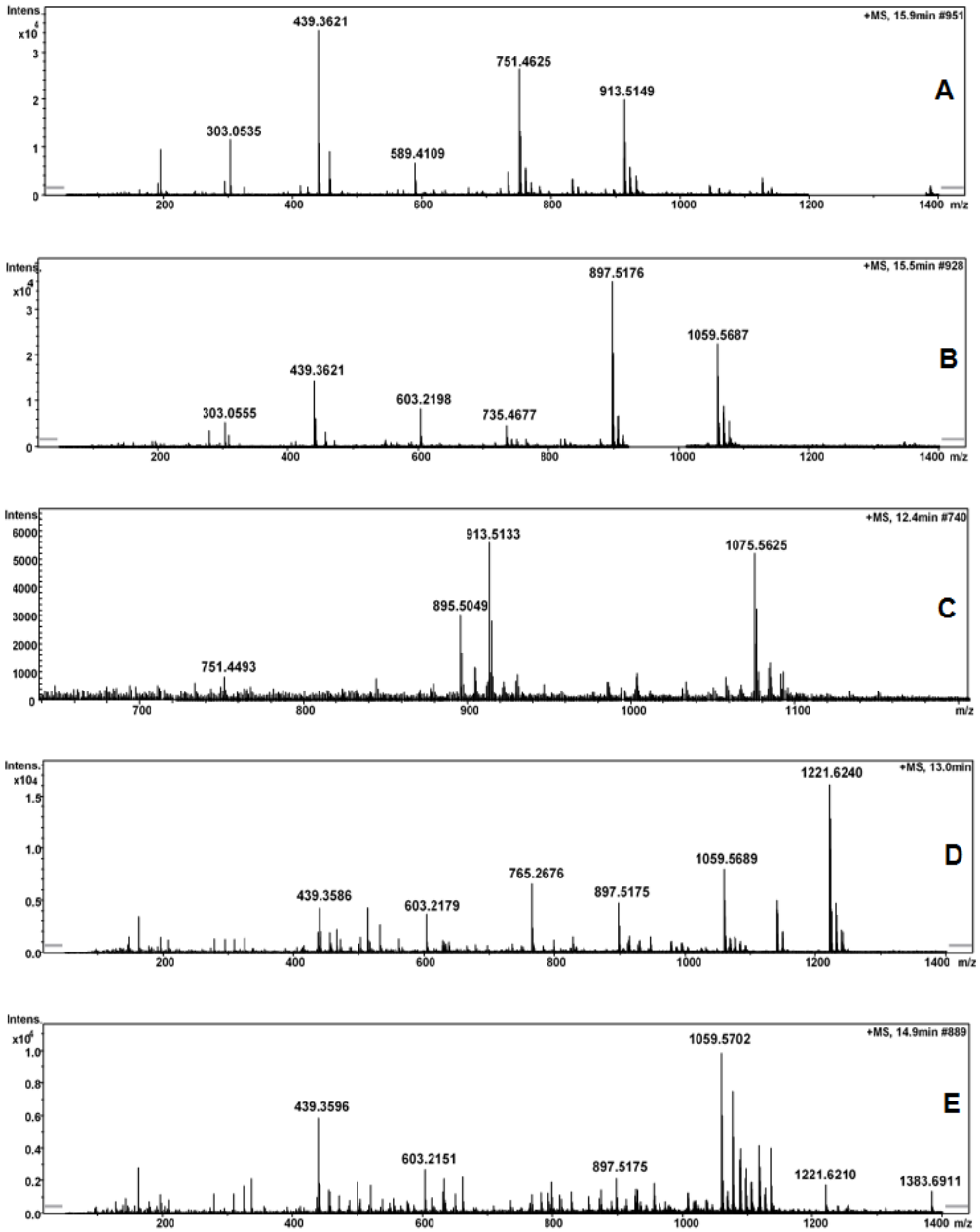


Figure 2- Spectra of ion fragments obtained from analysis of *yerba mate* (*Ilex paraguariensis*) extracts. Analysis performed by using ESI in positive ion mode. Ion fragments correspond to chemical compounds presented in **Table 1. (A)** Matesaponin **1** ($[M+H]^+$=913); **(B)** Matesaponin **2** ($[M+H]^+$=1059) **(C)** Matesaponin **3** ($[M+H]^+$=1075); **(D)** Matesaponin **4** ($[M+H]^+$=1221); **(E)** Matesaponin **5** ($[M+H]^+$=1383).

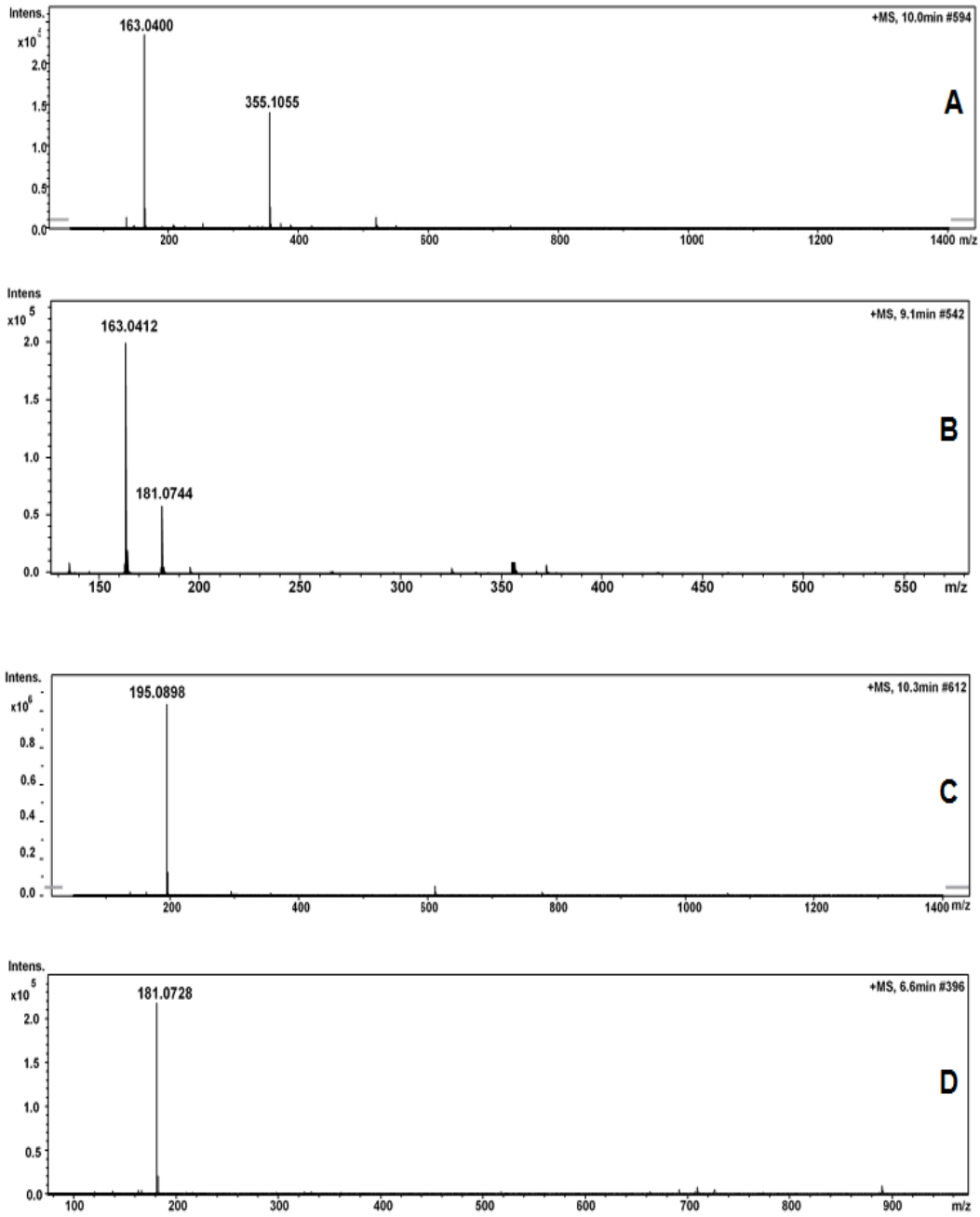


Figure 3- Spectra of ion fragments obtained from analysis of *yerba mate* (*Ilex paraguariensis*) extracts. Analysis performed by using ESI in positive ion mode. Ion fragments correspond to chemical compounds presented in **Table 1. (A)** Chlorogenic acid ($[M+H]^+$=355); **(B)** Caffeic acid ($[M+H]^+$=182); **(C)** Caffeine ($[M+H]^+$=195); **(D)** Theobromine ($[M+H]^+$=181).

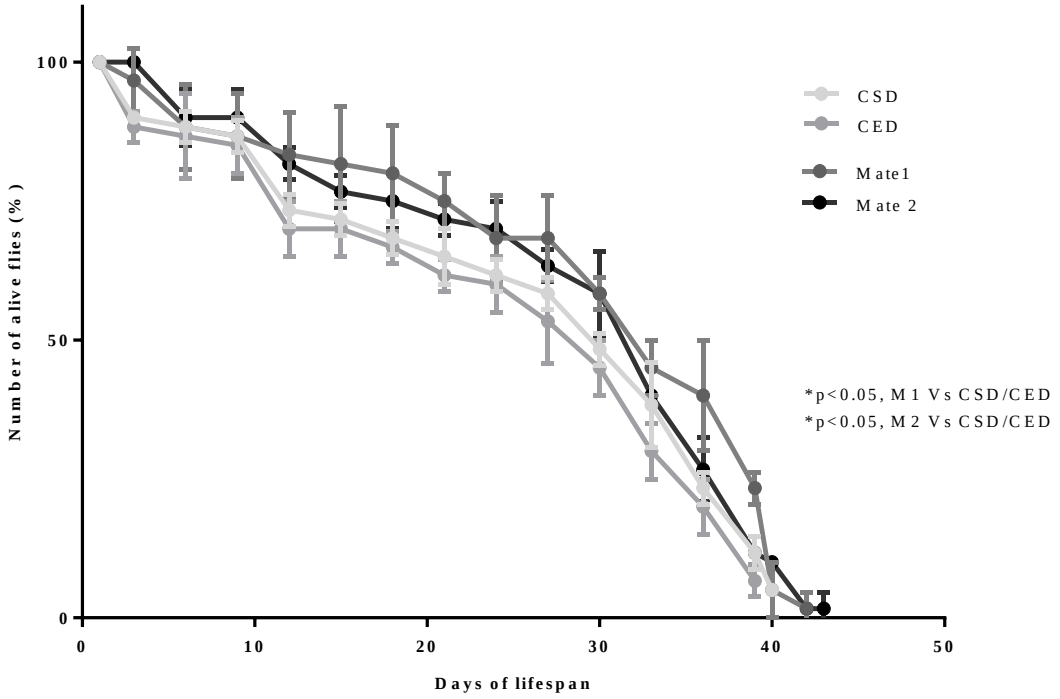
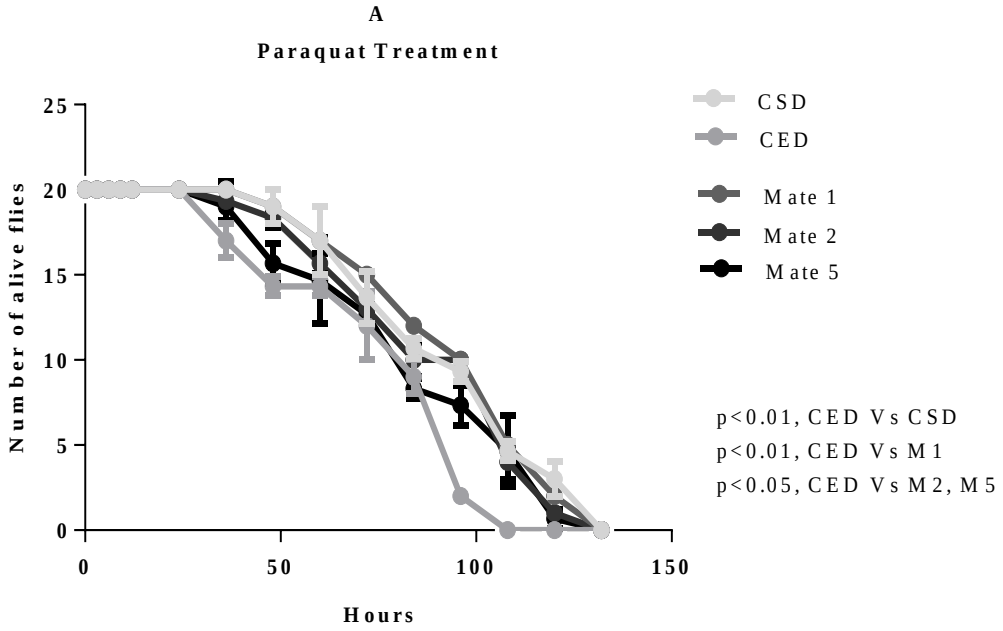
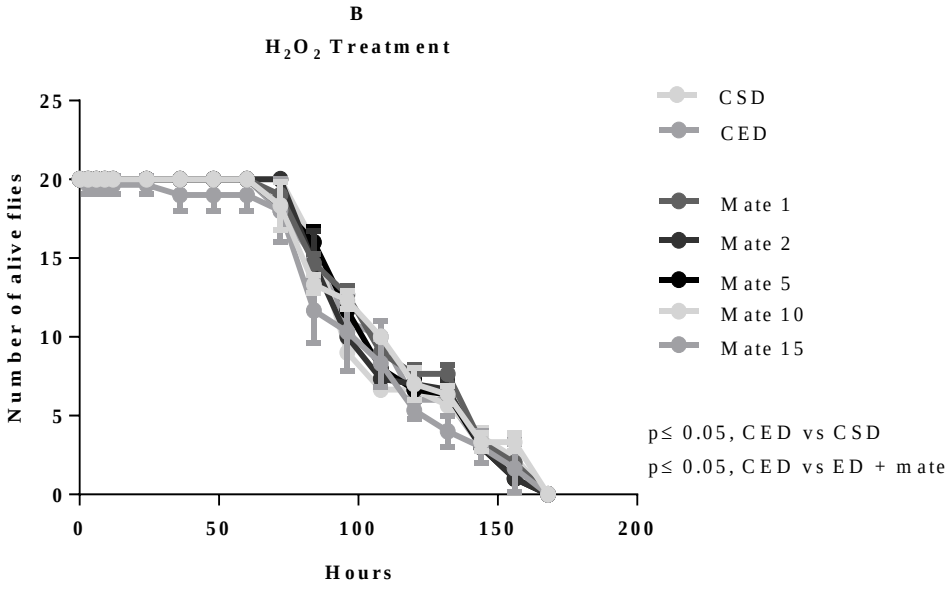


Figure 4- Lifespan curve of flies fed on a standard diet (CSD), experimental diet (CED) and experimental diet containing mates 1 and 2 (1 ml of extracts). The trials were made in triplicate and the data were expressed as the maximum lifespan of the last fly and mean lifespan for each group (there were 20 flies in each group). The Shapiro Wilk test showed that Mates 1 and 2 could significantly extend the mean lifespan of fruit flies ($p<0.05$).


A
Paraquat Treatment
Number of alive flies
Hours
0
5
10
15
20
25
0
50
100
150
CSD
CED
Mate 1
Mate 2
Mate 5
p<0.01, CED Vs CSD
p<0.01, CED Vs M1
p<0.05, CED Vs M2, M5


B
H2O2 Treatment
Number of alive flies
Hours
0
5
10
15
20
25
0
50
100
150
200
CSD
CED
Mate 1
Mate 2
Mate 5
Mate 10
Mate 15
p≤ 0.05, CED vs CSD
p≤ 0.05, CED vs ED + mate

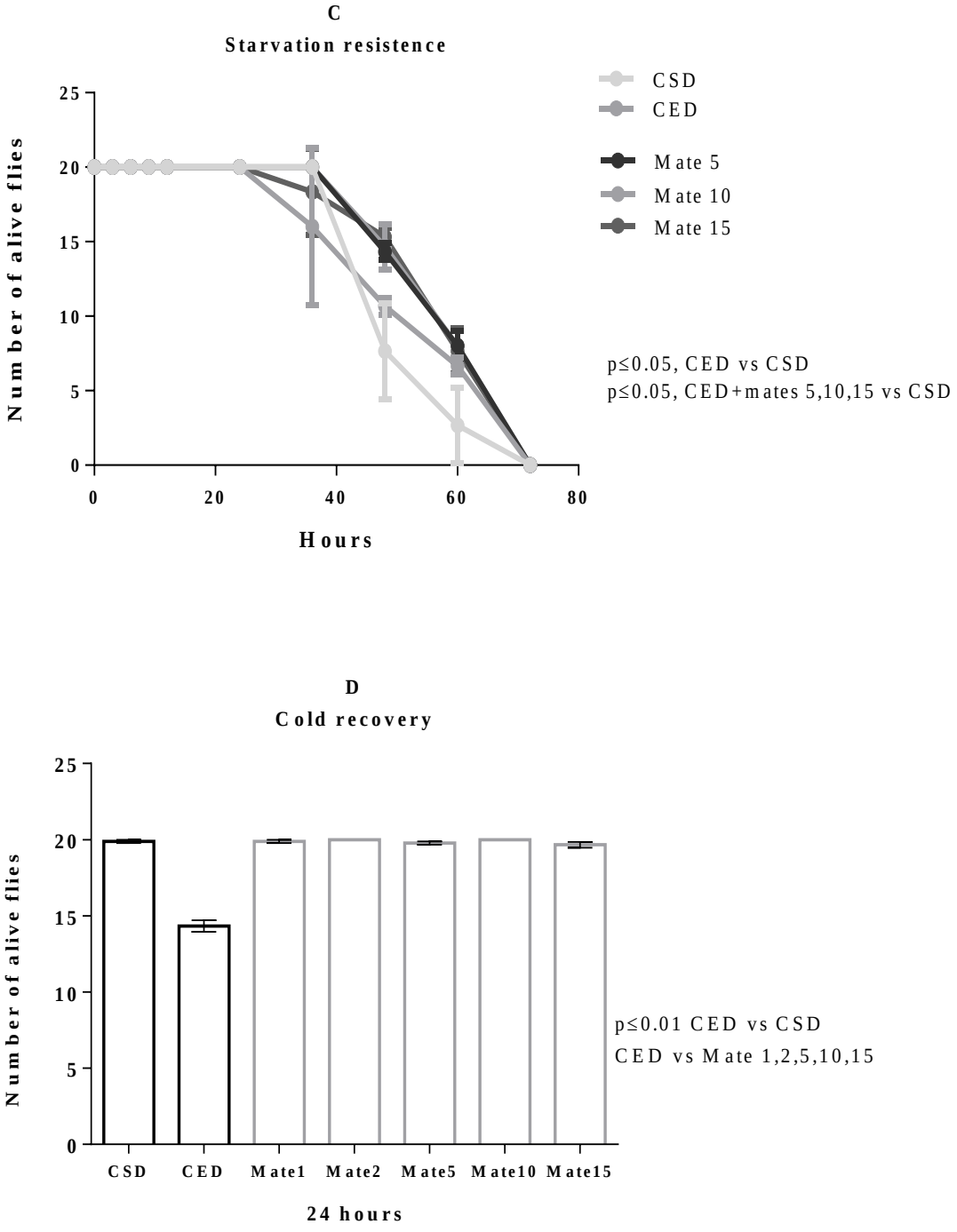


Figure 5- Diagram of the experimental paradigm for flies' resistance to paraquat treatment (A), hydrogen peroxide (B) treatment, starvation (C) and cold recovery (D). Flies were placed on SD and ED diets or ED+mates for 10 days; afterwards, they were stressed. *p<0.05 and **p<0.01.

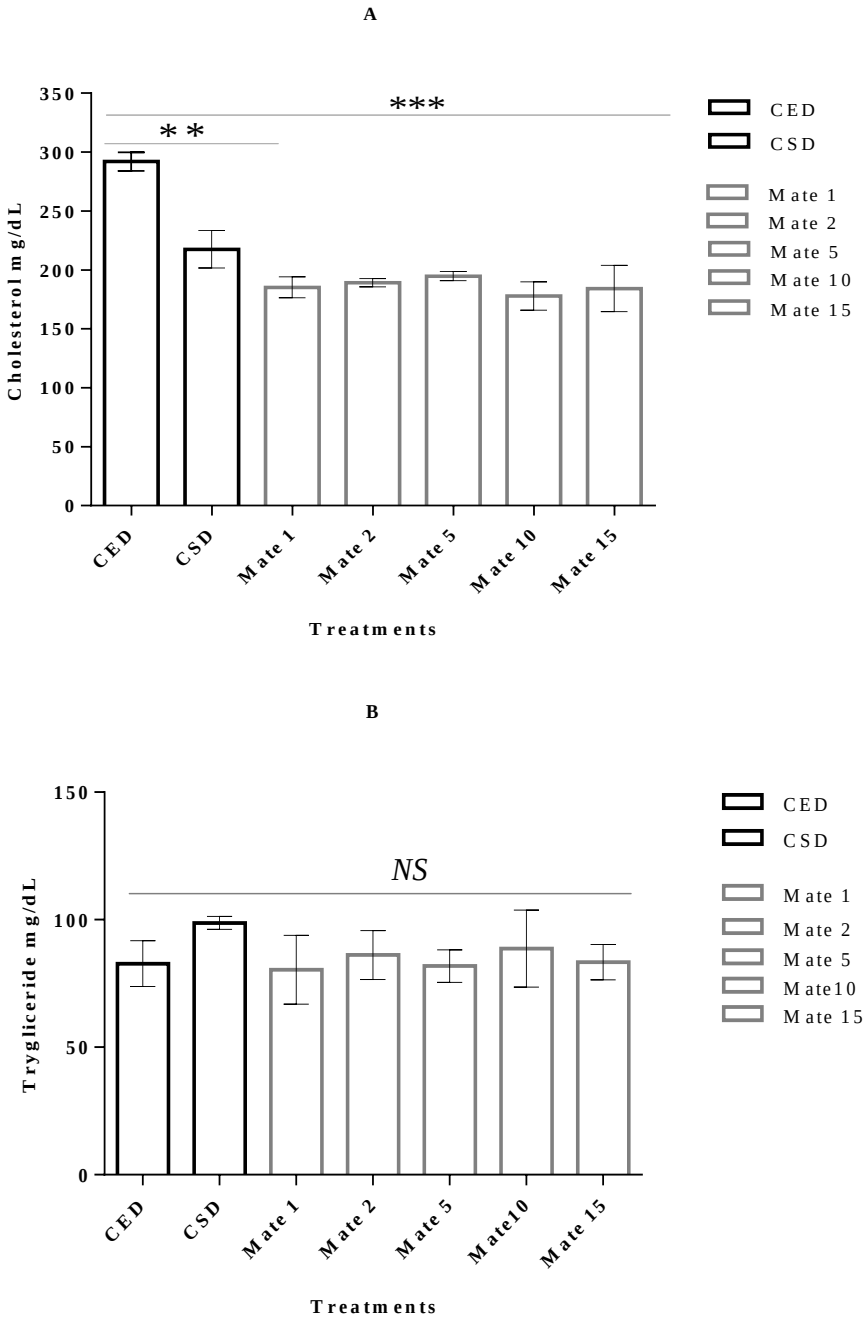


Figure 6- Cholesterol (A) and triglyceride (B) levels (per mg live weight) in the whole-body homogenate of flies after 10 days on various diets (standard, experimental and experimental+mates). Flies were homogenized in groups of 20 males. Error bars show the SEM * = $p<0.05$, *** = $p<0.001$, and NS= not significant.

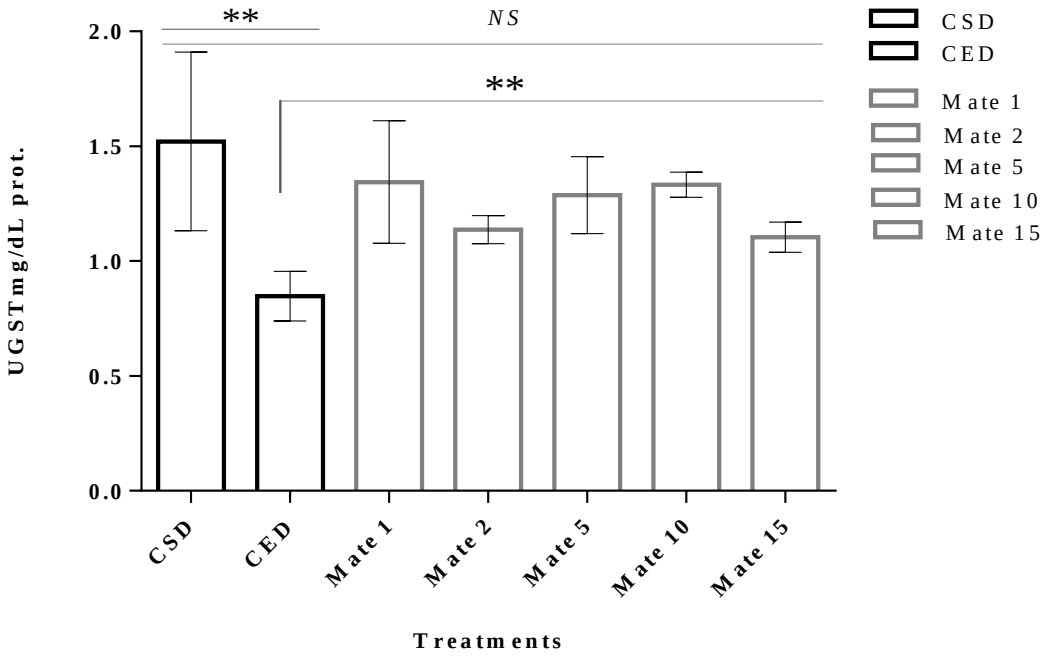


Figure 7- GST activity in the whole-body homogenate of flies after 10 days on various diets (standard, experimental and experimental+mates). Flies were homogenized in groups of 20 males. Error bars show the SEM ** = $p<0.01$, and NS= not significant.

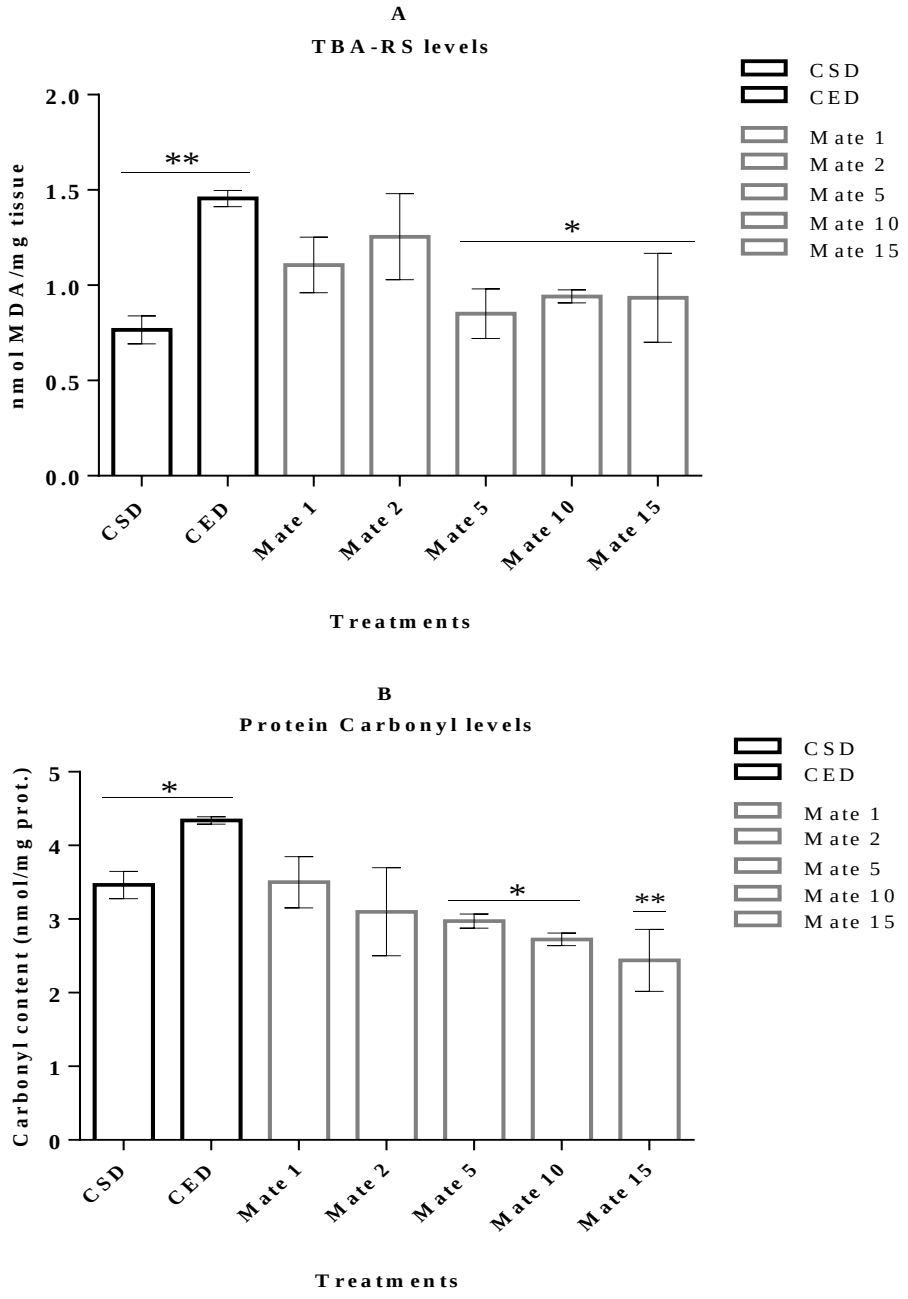


Figure 8- TBA-RS and carbonyl protein levels. Flies were homogenized in groups of 50 males. Error bars show the SEM * = $p<0.05$, ** = $p<0.01$ (n= 3). In (A) the values are expressed in nmol of MDA per mg of tissue. In (B) the values are expressed in nmol carbonyl/mg protein.

Supplementary material

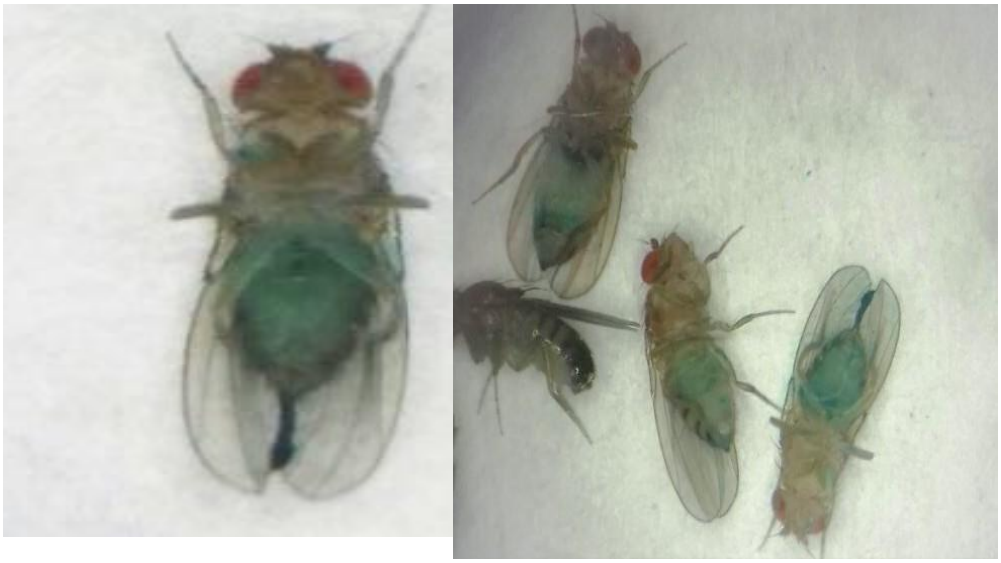


Figure 1- The intestinal flies' coloration. Evidence about flies' food intake, in accordance to Verspoor et al. (2015); doi: 10.3791/52645.

4.2 Artigo 2

Original research

Compounds from *Ilex paraguariensis* extracts confer antioxidant effects in the brains of rats subjected to chronic immobilization stress

Ana C. Colpo[a,b], Maria Eduarda de Lima[a,b], Marisol Maya-López[a], Hemerson Rosa[b], Cristina Márquez-Curiel[c], Sonia Galván-Arzate[c], Abel Santamaría[a*], Vanderlei Folmer[b]

[a]Laboratorio de Aminoácidos Excitadores, Instituto Nacional de Neurología y Neurocirugía, Mexico City, Mexico.

[b]Programa de Pós-Graduação em Bioquímica, Universidade Federal do Pampa (UNIPAMPA), Uruguaiana-RS, Brazil.

[c]Departamento de Neuroquímica, Instituto Nacional de Neurología y Neurocirugía, Mexico City, Mexico.

[*]Corresponding author: Abel Santamaría. Laboratorio de Aminoácidos Excitadores, Instituto Nacional de Neurología, Insurgentes Sur No.3877, México D.F. 14269, México. E-mail address: absada@yahoo.com

Abstract

Immobilization induces oxidative damage to the brain. *Ilex paraguariensis* extracts (Mate) and chlorogenic acid (CGA), its major natural compound, exert protective effects against reactive oxygen species (ROS) formation. Here, the effects of Mate and CGA on oxidative damage induced by chronic immobilization stress (CIS) in cortex (CTX), hippocampus (HIP) and striatum (STR) were investigated. For CIS, animals were immobilized during 6 h every day for 21 consecutive days. Rats received Mate or CGA daily by intra-gastric gavage 30 min before every restraint session. Endpoints of oxidative stress (levels of lipid peroxidation, protein carbonylation, and reduced (GSH) and oxidized (GSSG) forms of glutathione) were evaluated following CIS. While CIS increased oxidized lipids and carbonyl levels in all brain regions, CGA (and Mate in a lesser extent) attenuated lipid and protein oxidation as compared to control groups. GSH/GSSG balance showed a tendency to increase in all regions in response to stress and antioxidants. Taken together, our results support a protective role of dietary antioxidants against the neuronal consequences of stress.

Keywords *Yerba mate*; Chlorogenic acid; Immobilization stress; Oxidative damage; Antioxidant defense; Natural neuroprotective strategies.

Introduction

Chronic stress affects the functions of the nervous, endocrine and immune systems (Halliwell and Gutteride 2007). Stress elevates corticosterone (CORT) secretion, causing mitochondrial dysfunction induced by a prolonged decrease in the mitochondrial membrane potential (Takahashi et al. 2002), which results in excitotoxicity and reactive oxygen species (ROS) formation (Hansson et al. 2008). In addition, the increased levels of glucocorticoids produced during stress may affect the whole antioxidant capacity of the Central Nervous System (Şahin and Gümüşlü 2007). In line with this, restraint stress creates a neurotoxic environment in the brain, increasing the susceptibility of neuronal cells to suffer metabolic alterations and death through the activation of deleterious signaling pathways (Gerecke et al. 2013). Stress induced by immobilization is related with oxidative damage to lipids, proteins and DNA in the brain (Liu et al. 1996), possibly associated with mitochondrial dysfunction, disruption of energy metabolism, neuronal damage, impaired neurogenesis and induction of signaling events during apoptotic cell death (Grizzell et al. 2014), all accompanied by ROS production.

ROS and antioxidants have been the focus of a considerable number of current investigations. Polyphenols and other compounds from natural products have been described as major antioxidants. In this regard, *Ilex paraguariensis* extracts particularly rich in bioactive compounds, are known to possess various redox modulatory properties, the majority of which are associated with defense against ROS (Lima et al. 2014; Colpo et al. 2016).

Yerba mate is the main product obtained from branches and leaves of the *Ilex paraguariensis* tree. This product is used to make a traditional beverage consumed in South America, named "Chimarrão" in Brazil, "Mate" in Argentina and Uruguay, and

"Tererê" in Paraguay (Bracesco et al. 2011). The predominant compound of *yerba mate* extracts is chlorogenic acid (CGA, 3-O-caffeoylquinic acid) (Bracesco et al. 2011; Colpo et al. 2016). Recent evidence suggests that CGA may produce beneficial effects related to antioxidant activity (Gul et al. 2016; Liang and Kitts 2016). Additional evidence indicates that both *yerba mate* crude extracts and CGA can produce neuroprotective effects associated primarily to their activities as antioxidants. Gul et al. (2016) demonstrated that CGA protected rat cortical slices against H_2O_2-induced alterations on oxidative stress parameters. In turn, Lee et al. (2012) showed that CGA reduced brain damage, blood-brain barrier damage and brain edema by stimulating radical scavenging activity.

Regarding crude extracts, *Ilex paraguariensis* extracts have demonstrated the ability to prevent memory deficits in rats (Colpo et al. 2007; Santos et al. 2015), and a capacity to reduce the frequency of seizures, minimizing the neuronal damage associated with their periodic occurrence (Branco Cdos et al. 2013). Moreover, Cittadini et al. (2015) reported that this plant could be a source of chemopreventive agents to combat oxidative stress-related neurodegenerative pathologies because of its redox effects in brain regions.

Despite of the aforementioned evidence, the role of *yerba mate* crude extracts and CGA in modulating the oxidative stress-induced alterations in the brain remains unexplored. Thus, in this work we investigated whether *yerba mate* crude extracts and CGA may exert protective effects in the brains of rats submitted to CIS, and if these effects are due to their antioxidants capacities.

Materials and methods

Reagents and extracts preparation

Yerba mate was purchased in a popular market in Uruguay. The aqueous extract was prepared as an infusion of *Ilex paraguariensis* (Aquifoliaceae) at a 200 mg/ml concentration. The infusion was prepared with 10 ml of ultrapure water at 85ºC in 2 g of *yerba mate* for 10 min. The extract infusion was named simply "Mate". Chlorogenic acid (5.6 mM) was diluted in saline solution 0.9% (in accordance with the manufacturer's instructions). Thiobarbituric acid (TBA), HEPES, malondialdehyde (MDA), sucrose, and CGA were all obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other reagents were obtained as reagent-grade from other well-known commercial sources.

Animals

Thirty-nine adult male Wistar adult rats (225–250 g), which were not littermates, were obtained from the vivarium of the Instituto Nacional de Neurología y Neurocirugía (INNN- Mexico). Upon arrival at the INNN vivarium, the animals were housed in polycarbonate cages and had free access to food (rodent Chow 5001; PMI Feeds Inc., Richmond, IN, U.S.A.), water, and controlled conditions of temperature (25 ± 3 ° C), humidity (50 ± 10%), and a 12:12-h light-dark schedule. Rats were given four days to acclimate to the animal housing room after their arrival, and before any experimental manipulations took place. After the acclimation period, all animals were randomly assigned to one of six groups (n=6/group). All procedures were carried out in accordance to the guidelines established by the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health, and following the recommendations of the Ministry of Health of Mexico.

Chronic immobilization stress protocol and compounds administration design

Six experimental groups (n=6 rats per group randomly selected) were designed: Water, Water+CIS (Chronic Immobilization Stress), Mate, Mate+CIS, CGA, CGA+CIS. The groups submitted to CIS were immobilized daily during 6 h (from 8:00 am to 2:00 pm) for 21 consecutive days in a glass device with the following dimensions: 7 x 6 x 18 cm (Colín-González et al. 2015). To avoid the animals to adapt in the glass device, different daily stressing stimuli were applied for two hours during the restraint period; they included cold water basement, inclination of the box, hot water basement, ice, dark and light (all applied external to the glass device). Groups that were not submitted to CIS were isolated daily in acrylic cages (30 x 30 x 20 cm) during 6 h for 21 days. The four groups were deprived of food and water during the 6 h isolation-period. All groups received treatments by intra-gastric gavage, 30 min before immobilization or isolation for the same 21 days. The treatment dose was determined according to the body weight of the animal (1.5 µl/g for vehicle and mate; 0.5 µl/g for CGA) (Brasil 1996, 2004; Andrade et al. 2012; Lee et al. 2012). An additional small control group (n=3) was subjected to random stressors but not being immobilized; since these animals did not show changes in body weight, neither exhibited signs of anxiety, they only served to assess that random stressors *per se* exerted slight or no effects in control rats (data not represented).

At the end of the chronic stress procedure (21 days), all animals were euthanized by decapitation, their brains were extracted, and the cortex (CTX, as frontal cortex), hippocampus (HIP, as CA3) and striatum (STR, as caudate-putamen) were immediately dissected out. Tissue samples were frozen immediately after collected and kept frozen (-20^{0}C) until analysis.

Body weight

The body weight of animals was estimated individually using an analytical balance. This procedure was carried out every three days since the beginning of the experiment. In order to analyse the variations in this parameter, at the end of the treatment the final weight was subtracted from the initial weight.

Assay of lipid peroxidation

The assay to detect thiobarbituric acid-reactive substances (TBA-RS) was used for the determination of the levels of lipid peroxidation in tissue samples, according to a previous report (García et al. 2014). The principle of this technique is based on the reaction of TBA with oxidized lipids to form malonaldehyde (MDA) as a product of peroxidation, as well as other products. Results were corrected by the protein content in samples and expressed as nmol of TBA-reactive substances (TBAR-RS) per mg protein.

Assay of protein carbonylation

Hydrazone formation after reaction with DNPH was calculated as an index of protein carbonylation (Colín-González et al. 2013). The levels of proteins containing carbonyl groups were expressed as nmol of DNPH per mg of protein, using the molar absorption coefficient for DNPH (22.000 M^{-1} cm^{-1}). The total amount of protein was obtained through the estimation of the optical density at 280 nm in blank probes prepared in parallel. Results were expressed as the percent of increase vs. the control value.

Determination of oxidized and reduced glutathione concentrations

For measurement of GSH and GSSH levels, we used a method previously described by Galván-Arzate et al. (2005). The GSH and GSSG levels were determined in homogenates from the brain regions. Their fluorescent signals were recorded in a luminescence spectrometer at 420 nm of emission and 350 nm of excitation wavelengths (Perkin-Elmer LS50B equipment). For GSH levels, final results were expressed as nMol GSH per mg of protein. For GSSG levels, data were expressed as

nMol GSSG per mg of protein. The reduced/oxidized glutathione (GSH/GSSG) ratio was also calculated and expressed.

Statistical analysis

Data are presented as mean values ± one standard deviation (SD). All data were analyzed by two-way ANOVA followed by Tukey's or Bonferroni's tests, with a minimum level of significance of $p \leq 0.05$. The GraphPad Software, Inc. was used to create the artworks.

Results

Mate and chlorogenic acid prevented the chronic immobilization-induced body weight loss in stressed rats

Fig. 1 shows the changes in body weight of animals during the entire period of treatment in the different experimental groups. The body weight of each rat was recorded during the entire period of protocol involving immobilization. In rats subjected to Water+CIS (21 days), the weight loss was significant. The mean value obtained between the initial and final weights showed a loss of 16 grams in this experimental group ($p \leq 0.001$). In the groups submitted to CIS and treated with Mate or CGA there was no weight loss as compared to control group ($p \leq 0.01$ vs. CIS group).

Mate and chlorogenic acid prevented the chronic immobilization-induced changes in parameters of oxidative stress in rat brain regions

Lipid peroxidation

Results of TBA-RS formation in the cortex, hippocampus and striatum of control and stressed animals are presented in **Figs. 2a**, **2b**, and **2c**, respectively. In the cortex, CIS increased the levels of lipid peroxidation in the Water+CIS treated group by 35% ($p \leq 0.001$), and in the Mate+CIS treated group in 21.6% ($p \leq 0.05$), above their corresponding control groups. In the CIS+CGA treated group, there was no change in TBA-RS formation (4.33%) as compared to the control group (**Fig. 2a**).

Fig. 2b depicts the levels of TBA-RS formation in the hippocampus. There was a significant increase in this parameter in the Water+CIS treated group ($p \leq 0.01$), in which the level of lipid peroxidation was 98 % above the control group. Mate and CGA

significantly reduced the CIS-induced TBA-RS formation by 16 % and 8 %, respectively ($p \leq 0.001$).

The effect of CIS on the striatal levels of TBA-RS formation is showed in **Fig. 2c.** In this brain region, lipid peroxidation was found significantly increased in the Water+CIS ($p \leq 0.01$) and Mate+CIS ($p \leq 0.05$) treated groups, which corresponded to 33 % and 40 % above their corresponding controls. CGA was able to maintain the TBA-RS formation similar to control values.

When comparing the data of all groups submitted to CIS, it was found that, in the CTX and HIP, lipid peroxidation was significantly lower in the Mate- and the CGA-treated groups than the control (Water) treated group. In the STR, only CGA presented a significant response against stress. The statistical significance derived from the analysis of these data was represented by the letter "**c**" in **Fig. 2a**, **2b** and **2c**.

Protein carbonylation

The statistical analysis revealed that CIS condition produced a significant increase in protein oxidation in all three regions studied (**Figs. 3a, 3b and 3c**): using water as control, it was found that the protein carbonylation levels were increased by CIS in CTX, HIP and STR ($p \leq 0.001$). Mate was able to counteract the effects of CIS in the hippocampus ($p \leq 0.05$), whereas in the cortex and the striatum, this effect was not observed. In contrast, CGA was capable of inhibiting oxidative damage to proteins, as the carbonyl protein rate was similar to control in all the brain regions tested.

When comparing all groups submitted to CIS, the CGA-treated group exhibited lower levels of protein carbonylation in relation to the Water (control)-treated group in cortex ($p \leq 0.01$), hippocampus ($p \leq 0.05$) and striatum ($p \leq 0.001$). Mate significantly decreased the protein oxidation in the hippocampus ($p \leq 0.05$).

Glutathione (GSH/GSSG) balance

Table 1 shows the changes in GSH, GSSG and GSH/GSSG balance in control and treated groups. Except for the significant increase of GSH levels in the cortex of the CIS group compared with control group ($p \leq 0.05$), no other changes in GSH, GSSG of GSH/GSSG values were found among groups, although the three regions analyzed showed marked tendencies to increase the GSH/GSSG balance in CIS, Mate+CIS and CGA+CIS conditions, suggesting that GSH defense is activated under stressing conditions in an attempt to counteract oxidative stimuli.

Discussion

In this report, we have evaluated for the first time the effects of *yerba mate* extract, and its major compound CGA, on stress induced by immobilization in rats. Particular emphasis was given to oxidative stress and the redox status of the brain. Our results confirm that CIS produces oxidative damage to specific brain regions, affecting important functions of the CNS.

Noteworthy, it is known that many factors may modify the responses to stress during chronic immobilization conditions, including the predictability to stressors. In this regard, Thakur et al. (2015) suggested that chronic unpredictable stress responses, and its unexpected nature, prevent the development of adaptation and coping strategies, thus accounting for a better model of CIS. Because of this, in the present study we choose to apply different types of stressors in our stress model.

Chronic stress is associated with dysregulation of energy homeostasis; previous reports have demonstrated that chronic exposure to restraint stress reduces the body weight of rodents (Gamaro et al. 2003; Flak et al. 2011). Our study confirms that CIS was responsible for a significant weight loss, while mate and CGA were able to reduce the alterations associated to the stress mechanisms, thus maintaining homeostasis and limiting the weight loss.

The factors associated to these positive effects remain unclear; however, it is known that physical and psychological stressors cause oxidative damage by inducing an imbalance between the pro-oxidant and antioxidant status (Zafir and Banu 2009). Stress is known to induce alterations in the energetic metabolism, consequently leading to the formation of ROS, such as superoxide radical, from the mitochondrial electron

transport chain (Du et al. 2009). The role of polyphenols in the animals' body weight gain can be associated, at least in part, with the improvement in the redox status and an improved systemic response to the induced stress. However, we need to extend this knowledge to fully understand the action of the compounds from mate in food intake-related genes, as well as in the regulation of the stress response through corticosterone.

With respect to the brain sensitivity to alterations in redox status, this condition occurs mainly because the brain is an important oxygen consumer. However, the presence of excitatory amino acids, its modest capacity to activate antioxidant defence systems, and many other factors, are pointed out as relevant for its increased susceptibility to oxidative cell damage (Halliwell and Gutteride 2007). Moreover, neuronal cells are vulnerable to the attack of ROS mainly because neuronal membranes are rich in polyunsaturated fatty acids (PUFA) (Friedman 2010). Disorders of cellular metabolic homeostasis resulting from enhanced levels of ROS, glucose or reactive carbonyl compounds, cause modifications on cellular components, particularly proteins (Ambrożewicz and Bielawska 2016).

Commonly, oxidative damage is manifested as an increase in lipid peroxidation, DNA base oxidation products, and oxidative protein damage (Halliwell 2001), and these events have been increasingly associated to neurodegenerative disorders (Chen et al. 2012). For instance, the lipid peroxidation end-product 4-hydroxy-2,3-nonenal (HNE) is cytotoxic to neurons and impairs the function of membranes, including the neuronal glucose transport and use involving the inactivation α-ketoglutarate dehydrogenase (an enzyme with relevant activity in the cycle of tricarboxylic acids) (Bruce-Keller et al. 1998; Mclain et al. 2011). In turn, ROS-mediated protein oxidation produces secondary damage to other biomolecules, for example by raising the intracellular Ca^{2+} levels (Halliwell and Gutteride 2007).

Responses to immobilization stress result in the over-production of free radicals, leading to lipid peroxidation in cell membranes (Şahin and Gümüşlü 2007). The present study clearly demonstrates the CIS-induced oxidative damage in CTX, HIP and STR, evidenced by the increase of TBA-RS and carbonyl protein levels. In contrast, treatment of animals with CGA resulted in a decrease in these parameters, in comparison to the untreated stressed animals. Mate, in turn, was able to reduce the oxidative damage to lipids only in HIP. This finding suggests that CGA is more efficient as an isolated antioxidant than Mate, to counteract oxidative damage in the brain in this restraint stress model.

Natural antioxidants prevent the ROS generation, oxidation of proteins, and lipid peroxidation, thus acting as upstream therapeutic barriers to oxidative stress (Chen et al. 2012). Results of this study showed that hippocampal cells display better responses to the stimulus produced by *yerba mate* crude extracts on antioxidant activity than cortex and striatum. In turn, these differences could be due to intrinsic characteristics of the hippocampal cells, especially regarding their properties to use external antioxidant sources. These characteristics deserve further and detailed investigation.

In addition, some studies have reported *yerba mate* to possess neuroprotective properties, such as antidepressant-like (Ludka et al. 2016), anxiolytic and stimulant (Santos et al. 2015) effects, as well as attenuation of dyskinesia and memory dysfunction (Colpo et al. 2007; Costa et al. 2015), and reduction of the frequency of pentylenetetrazol-induced seizures (Branco Cdos et al. 2013). These effects emphasize the therapeutic potential of this natural product.

CGA is a free radical and metal scavenger and represents the major component in *Ilex paraguariensis* extracts (Colpo et al. 2016). In the brain, CGA has shown to improve the spatial learning and memory (Han et al. 2010), also exerting anti-amnesic

activity via inhibition of acetylcholinesterase and malondialdehyde (MDA) in the hippocampus and frontal cortex (Kwon et al. 2010). CGA also reduces brain and blood-brain barrier (BBB) damages, as well as brain edema, by radical scavenging activity and inhibitory effects on metalloproteinases (Lee et al. 2012). The present report highlights the protective effects of CGA in different brain regions from rats submitted to CIS. Of note, the brain concentration of bioactive compounds uptake is limited, although it is known that, usually, metabolites are accumulated at levels around 0.4 nmol/g tissue (El-Mohsen et al. 2006). Numerous mechanisms, such as free radical scavenging, metal chelation and the modulation of enzyme activities, have been proposed to explain the positive impact of polyphenols in the brain (Schaffer et al. 2012). Furthermore, Halliwell (2006) observed that the sufficient supply of the CNS with antioxidants is of prime importance because of the brain's vulnerability to oxidative and nitrosative stress.

Antioxidant enzymes play important roles in the cerebral cellular defense against oxidative damage, and are able to lower the risk of some neurological disorders (Halliwell and Gutteride 2007). Reduced glutathione (GSH) is involved in many metabolic processes and prevent protein-SH groups from oxidation and cross-linking (Halliwell and Gutteride 2007). Moreover, GSH plays a fundamental role in the detoxification of ROS, which is critical for the normal function of the brain (Hirrlinger et al. 2002). During oxidative stress, GSSG and glutathione S-conjugates are generated, being GSSG/2GSH described as an important indicator of the intracellular redox environment (Khramtsov and Gillies 2014). Our results show that CIS produced an increase in GSH levels only in CTX ($p \leq 0.05$), and this change can be interpreted as a more adaptive/compensatory response to the oxidative damage in course. The same

interpretation can be adopted for the tendency induced by CIS, CIS+Mate and CIS+CGA conditions to increase the GSH/GSSG balance in the three regions studied.

Consistent with a cytoprotective role, low GSH levels decrease cellular antioxidant capacity, as occurs in various neurodegenerative disorders. In turn, elevated GSH levels and GSH/GSSG balance increase antioxidant capacity and resistance to oxidative stress (Ballatori et al. 2009). The mechanisms by which GSH is increased remain unclear since they might be due to a variety of factors, including increased transcriptional activity of proteins used to synthesize GSH, increased translational activity, decreased degradation of GSH, increased reduction of GSSG, and increased transport of precursors (Johnson et al. 2012).

There is now enough collected evidence to claim beneficial effects of antioxidants in prolonged stress and unbalanced redox present in neurodegenerative disorders, such as Alzheimer's (AD) and Parkinson's (PD) diseases. For instance, Zaidi and Banu (2004) showed that Vitamin E was effective in restoring antioxidant systems in the brain tissue of animals submitted to restraint stress. In addition, in the hippocampus, S-allyl cysteine (SAC), the most abundant organosulfur molecule found in aged garlic extracts, exerted a modulatory role on antioxidant responses in acute restraint stress (Colín-González et al. 2015). Moreover, Hong et al. (2014) demonstrated that *Rooibos tea* prevents lipid peroxidation, restores stress-induced protein degradation, and regulates GSH metabolism. Our findings are in agreement with all these reports. Since the beneficial effects exerted by Mate and CGA are assumed to be due to their properties as polyphenols, then the transfer of hydrogen atoms, single electron transfer and metal chelation are likely to be part of their protective effects observed here (Leopoldini et al. 2011). Furthermore, Ríos-Hoyo et al. (2014) observed that polyphenols could evoke

beneficial effects acting through the activation of metabolic pathways, a consideration that shall be investigated for the model developed here in a near future.

To conclude, in the present study, we evidenced a pro-oxidant action of CIS in rat brain regions. It was also demonstrated that mate and CGA presented antioxidant capacity in this stress model. Because ROS are involved in chronic stress and degenerative disorders, the knowledge about natural compounds counteracting the harmful effects of these disorders is an important contribution.

Ethical approval

The care and handling of all animals was carried out in accordance with the guidelines of the Guide of the National Institutes of Health for the Care and Use of Laboratory Animals NOM-062-ZOO 1999, as well as the Local Committee of Bioethics of the Instituto Nacional de Neurología and Neurocirugía (Mexico) established upon approval of the Ministry of Health of Mexico.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

AC Colpo, ME de Lima, M Maya-López, H Rosa and S Galván-Arzate, all contributed to project development, data collection and analysis. AC Colpo and A Santamaría contributed to writing of the manuscript. AC Colpo, V Folmer and A Santamaría designed the whole project. All authors have approved the final manuscript.

Funding

This work was supported by CAPES (Grant No. 99999.006340/2015-06, A.C.), UNIPAMPA (A.C., Brazil) and INNN (A.S., Mexico).

Acknowledgement

The authors wish to thank Drs. Mina Königsberg, Viridiana Yazmín González-Puertos and Pedro Posadas-Rodríguez for their valuable technical support.

References

Ambrożewicz, E., and Bielawska, K. 2016. Protein carbonylation - reasons, effects and determination. Postepy Biochem. **62:** 495-505. PMID:28132451.

Andrade, J.P., and Assunção, M. 2012. Protective effects of chronic green tea consumption on age-related neurodegeneration. Curr. Pharm. Des. **18:** 4-14. doi: 10.2174/138161212798918986. PMID:22211685.

Ballatori, N., Krance, S.M., Notenboom, S., Shi, S., Tieu, K., and Hammond, C.L. 2009. Glutathione dysregulation and the etiology and progression of human diseases. Biol. Chem. **390:** 191-214. doi:10.1515/BC.2009.033. PMID:19166318.

Bracesco, N., Sanchez, A.G., Contreras, V., Menini, T., and Gugliucci, A. 2011. Recent advances on *Ilex paraguariensis* research: Minireview. J. Ethnopharmacol. **136:** 378-384. doi:10.1016/j.jep.2010.06.032. PMID:20599603.

Branco Cdos, S., Scola, G., Rodrigues, A.D., Cesio, V., Laprovitera, M., Heinzen, H., et al. 2013. Anticonvulsant, neuroprotective and behavioral effects of organic and conventional yerba mate (*Ilex paraguariensis* St. Hil.) on pentylenetetrazol-induced seizures in Wistar rats. Brain Res. Bull. **92**: 60-68. doi:10.1016/j.brainresbull.2012.11.008. PMID:23270878.

Brasil. 1996. Agência Nacional de Vigilância Sanitária. Resolução- n. 116 – Normas para estudo da toxicidade de produtos fitoterápicos, de 08/08/1996. Diário Oficial da República Federativa do Brasil, Poder Executivo, Brasília, DF.

Brasil. 2004. Agência Nacional de Vigilância Sanitária. Resolução - Guia para a realização de estudos de toxicidade pré-clínica de fitoterápicos, de 16/03/2004. Diário Oficial da República Federativa do Brasil, Poder Executivo, Brasília, DF.

Bruce-Keller, A.J., Li, Y.J., Lovell, M.A. Kraemer, P.J., Gary, D.S., Brown, R.R., et al. 1998. 4-Hydroxynonenal, a product of lipid peroxidation, damages cholinergic neurons and impairs visuospatial memory in rats. J. Neuropathol. Exp. Neurol. **57:** 257-267. PMID:9600218.

Chen, X., Guo, C., and Kong, J. 2012. Oxidative stress in neurodegenerative diseases. Neural Regen. Res. **7:** 376-385. doi:10.3969/j.issn.1673-5374.2012.05.009. PMID:25774178.

Cittadini, M.C., Canalis, A.M., Albrecht, C., and Soria, E.A. 2015. Effects of oral phytoextract intake on phenolic concentration and redox homeostasis in murine encephalic regions. Nutr. Neurosci. **18:** 316-322. doi:10.1179/1476830514Y.0000000130. PMID:24840738.

Colín-González, A.L., Becerril, H., Flores-Reyes, B.R. Torres, I., Pinzón, E., Santamaría-Del Angel, D., et al. 2015. Acute restraint stress reduces hippocampal oxidative damage and behavior in rats: Effect of S-allyl cysteine. Life Sci. **135:** 165-172. doi:10.1016/j.lfs.2015.06.009. PMID:26135627.

Colín-González, A.L., Orozco-Ibarra, M., Chánez-Cárdenas, M.E., Rangel-López E., Santamaría, A., Pedraza-Chaverri, J., et al. 2013. Heme oxygenase-1 (HO-1) upregulation delays morphological and oxidative damage induced in an excitotoxic/pro-oxidant model in the rat striatum. Neuroscience, **231:** 91-101. doi:10.1016/j.neuroscience.2012.11.031. PMID:23201824.

Colpo, A.C., Rosa, H., Lima, M.E., Pazzini, C.E., de Camargo, V.B., Bassante, F.E., et al. 2016. Yerba mate (*Ilex paraguariensis* St. Hill.)-based beverages: How

successive extraction influences the extract composition and its capacity to chelate iron and scavenge free radicals. Food Chem. **209:** 185-195. doi:10.1016/j.foodchem.2016.04.059. PMID:27173551.

Colpo, G., Trevisol, F., Teixeira, A.M. Fachinetto, R., Pereira, R.P., Athayde, M.L., et al. 2007. *Ilex paraguariensis* has antioxidant potential and attenuates haloperidol-induced orofacial dyskinesia and memory dysfunction in rats. Neurotox. Res. **12:** 171-180. PMID:17967741.

Costa, P., Gonçalves, J.F., Baldissarelli, J., Mann, T.R., Abdalla, F.H., Fiorenza, A.M., et al. 2015. Curcumin attenuates memory deficits and the impairment of cholinergic and purinergic signaling in rats chronically exposed to cadmium. Environ. Toxicol. **32:** 70-83. doi:10.1002/tox.22213. PMID:26592365.

Du, J., Wang, Y., Hunter, R., Wei, Y., Blumenthal, R., Falke, C., et al. 2009. Dynamic regulation of mitochondrial function by glucocorticoids. Proc. Natl. Acad. Sci. **106:** 3543-3548. doi:10.1073/pnas.0812671106. PMID:19202080.

El-Mohsen, M.A., Bayele, H., and Kuhnle, G. 2006. Distribution of [3H] trans-resveratrol in rat tissues following oral administration. Br. J. Nutr. **96:** 62-70. PMID:16441916.

Flak, J.N., Jankord, R., Solomon, M.B., Krause, E.G., and Herman, J.P. 2011. Opposing effects of chronic stress and weight restriction on cardiovascular, neuroendocrine and metabolic function. Physiol. Behav. **104:** 228-234. doi:10.1016/j.physbeh.2011.03.002. PMID:21396386.

Friedman, J. 2010. Oxidative stress and free radical damage in neurology (why is the Nervous System vulnerable to oxidative stress?). Springer New York, pp. 19-27.

Galván-Arzate, S., Pedraza-Chaverri, J., Medina-Campos, O.N., Maldonado, P.D., Vázquez-Román, B., Ríos, C., et al. 2005. Delayed effects of thallium in rat brain:

regional changes in lipid peroxidation and behavioral markers, but moderate alterations in antioxidants, after a single administration. Food Chem. Toxicol. **43:** 1037-1045. doi:10.1016/j.fct.2005.02.006. PMID:15833379.

Gamaro, G.D., Manoli, L.P., Torres, I.L. Silveira, R., and Dalmaz, C. 2003. Effects of chronic variate stress on feeding behavior and on monoamine levels in different rat brain structures. Neurochem. Int. **42:** 107-111. PMID:12421590.

García, E., Santana-Martínez, R., Silva-Islas, C.A. Colín-González, A.L., Galván-Arzate, S., Heras, Y., et al. 2014. S-allyl cysteine protects against MPTP-induced striatal and nigral oxidative neurotoxicity in mice: participation of Nrf2. Free Radic. Res. **48:** 159-167. doi:10.3109/10715762.2013.857019. PMID:24147739.

Gerecke, K.M., Kolobova, A., Allen, S., and Fawer, J.L. 2013. Exercise protects against chronic restraint stress-induced oxidative stress in the cortex and hippocampus. Brain Res. **1509:** 66-78. doi:10.1016/j.brainres.2013.02.027. PMID:23499928.

Grizzell, J.A., Iarkova, A., Holmes, R. Mori, T., and Echeverria, V. 2014. Cotinine reduces depressive-like behavior, working memory deficits, and synaptic loss associated with chronic stress in mice. Behav. Brain Res. **268:** 55-65. doi:10.1016/j.bbr.2014.03.047. PMID:24713149.

Gul, Z., Demircan, C., Bagdas, D., and Buyukuysal, E.L. 2016. Protective effects of chlorogenic acid and its metabolites on hydrogen peroxide-induced alterations in rat brain slices: a comparative study with resveratrol. Neurochem. Res. **41:** 2075-2085. doi:10.1007/s11064-016-1919-8. PMID:27161374.

Halliwell, B. 2001. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs Aging, **18:** 685-716. PMID:11599635.

Halliwell, B. 2006. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiol. **141:** 312-322. doi:10.1104/pp.106.077073. PMID:16760481.

Halliwell, B., and Gutteride, J.M.C. 2007. Free Radicals in Biology and Medicine. Ed. 4, New York, 581 pp.

Han, J., Miyamae, Y., Shigemori, H., and Isoda, H. 2010. Neuroprotective effect of 3,5-di-o-caffeoylquinic acid on SH-SY5Y cells and senescence-accelerated-prone mice 8 through the up-regulation of phosphoglycertate kinase 1. Neuroscience, **169:**1039-1045. doi:10.1016/j.neuroscience.2010.05.049. PMID:20570715.

Hansson, M.J., Månsson, R., Morota, S., Uchino, H., Kallur, T., Sumi, T., et al. 2008. Calcium-induced generation of reactive oxygen species in brain mitochondria is mediated by permeability transition. Free Radic. Biol. Med. **45:** 284-294. doi: 10.1016/j.freeradbiomed.2008.04.021. PMID:18466779.

Hirrlinger, J., Schulz, J.B., and Dringen, R. 2002. Glutathione release from cultured brain cells: multidrug resistance protein 1 mediates the release of GSH from rat astroglial cells. J. Neurosci. Res. **69:** 318-326. doi:10.1002/jnr.10308. PMID:12125073.

Hong, I.S., Lee, H.Y., and Kim, H.P. 2014. Anti-oxidative effects of Rooibos tea (*Aspalathus linearis*) on immobilization-induced oxidative stress in rat brain. PLoS One, **10:** e0142287. doi:10.1371/journal.pone.0087061. PMID:24466326.

Johnson, W.M., Wilson-Delfosse, A.L., and Mieyal, J.J. 2012. Dysregulation of glutathione homeostasis in neurodegenerative diseases. Nutrients, **4:** 1399-1440. doi:10.3390/nu4101399. PMID:23201762.

Khramtsov, V.V., and Gillies, R.J. 2014. Janus-faced tumor microenvironment and redox. Antioxid. Redox Signal. **21:** 723-729. doi:10.1089/ars.2014.5864. PMID:24512276.

Kwon, S.H., Lee, H.K., Kim, J.A. Hong, S., Kim, H.C., Jo, T.H., et al. 2010. Neuroprotective effects of chlorogenic acid on scopolamine induced amnesia via anti-acetylcholinesterase and anti-oxidative activities in mice. Eur. J. Pharmacol. **649**: 210-217. doi:10.1016/j.ejphar.2010.09.001. PMID:20854806.

Lee, K., Lee, J.S., Jang, H.J., Kim, S.M., Chang, M.S., Park, S.H., et al. 2012. Chlorogenic acid ameliorates brain damage and edema by inhibiting matrixmetalloproteinase-2 and 9 in a rat model of focal cerebral ischemia. Eur. J. Pharm. **689:** 89-95. doi:10.1016/j.ejphar.2012.05.028. PMID:22659584.

Leopoldini, M., Russo, N., and Toscano, M. 2011. The molecular basis of working mechanism of natural polyphenolic antioxidants. Food Chem. **125:** 288-306. doi:10.1016/j.foodchem.2010.08.012.

Liang, N., and Kitts, D.D. 2016. Role of chlorogenic acids in controlling oxidative and inflammatory stress conditions. Nutrients, **25:** 8. doi:10.3390/nu8010016. PMID:26712785.

Lima, M.E., Colpo, A.C., Salgueiro, W.G., Sardinha, G.E., Avila, D.S., and Folmer, V. 2014. *Ilex paraguariensis* extract increases lifespan and protects against the toxic effects caused by paraquat in *Caenorhabditis elegans*. Int. J. Environ. Res. Public Health. **11:** 10091-10104. doi:10.3390/ijerph111010091. PMID:25264684.

Liu, J., Wang, X., Shigenaga, M.K., Yeo, H.C., Mori, A., and Ames, B.N. 1996. Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. FASEB J. 10: 1532-1538. PMID:8940299.

Ludka, F.K., Tandler, L.F., Kuminek, G., Olescowicz, G., Jacobsen, J., and Molz, S. 2016. *Ilex paraguariensis* hydroalcoholic extract exerts antidepressant-like and neuroprotective effects: involvement of the NMDA receptor and the L-arginine-NO pathway. Behav. Pharmacol. **27:** 384-392. doi:10.1097/FBP.0000000000000211. PMID:26703367.

McLain, A.L., Szweda, P.A., and Szweda, L.I. 2011. α-Ketoglutarate dehydrogenase: A mitochondrial redox sensor. Free Radic. Res. **45:** 29-36. doi:10.3109/10715762.2010.534163. PMID:21110783.

Ríos-Hoyo, A., Cortés, M.J., Ríos-Ontiveros, H., Meaney, E., Ceballos, G., and Gutiérrez-Salmeán, G. 2014. Obesity, metabolic syndrome, and dietary therapeutical approaches with a special focus on nutraceuticals (polyphenols): a mini-review. Int. J. Vitam. Nutr. Res. **84:** 113-123. doi:10.1024/0300-9831/a000198. PMID:26098475.

Sahin, E., and Gümüşlü, S. 2007. Immobilization stress in rat tissues: Alterations in protein oxidation, lipid peroxidation and antioxidant defense system. Comp. Biochem. Phys. C. **144:** 342-347. doi:10.1016/j.cbpc.2006.10.009. PMID:17157074.

Santos, E.C., Bicca, M.A., Blum-Silva, C.H., Costa, A.P., Dos Santos, A.A., Schenkel, E.P., et al. 2015. Anxiolytic-like, stimulant and neuroprotective effects of *Ilex paraguariensis* extracts in mice. Neuroscience, **292:** 13-21. doi:0.1016/j.neuroscience.2015.02.004. PMID:25681522.

Schaffer, S., and Halliwell, B. 2012. Do polyphenols enter the brain and does it matter? Some theoretical and practical considerations. Genes Nutr. **27:** 505. doi: 10.1007/s12263-011-0255-5. PMID:22012276.

Takahashi, T., Kimoto, T., Tanabe, N., Hattori, T.A., Yasumatsu, N., and Kawato, S. 2002. Corticosterone acutely prolonged N-methyl-D-aspartate receptor-mediated

Ca2+ elevation in cultured rat hippocampal neurons. J. Neurochem. **83:** 1441-1451. PMID:12472898.

Thakur, T., Anand, R., Ray, A., and Gulati, K. 2015. Differential effects of chronic predictable and unpredictable stress on neurobehavioral and biochemical responses in rats. Ther. Targets Neurol. Dis. **2:** e603. doi:10.14800/ttnd.603.

Zafir, A., and Banu, N. 2009. Modulation of in vivo oxidative status by exogenous corticosterone and restraint stress in rats. Stress, **12:** 167-177. doi:10.1080/10253890802234168. PMID:18850490.

Zaidi, S.M., and Banu, N. 2004. Antioxidant potential of vitamins A, E and C in modulating oxidative stress in rat brain. Clin Chim Acta, **340:** 229-33. PMID:14734217.

Table 1. Status of glutathione balance in control and Mate- or CGA-treated groups after 21 days of CIS.

	Parameters	Water	Water+CIS	Mate	Mate+CIS	CGA	CGA+CIS
CTX	GSH (nmol/mg protein)	0.57±0.03	0.92±0.28*	0.50±0.03	0.48±0.09	0.52±0.01	0.52±0.14
	GSSG (nmol/mg protein)	2.04±0.62	2.32±0.79	1.58±0.30	1.03±0.31	1.98±0.38	1.27±0.38
	GSH/GSSG (redox status)	0.29±0.08	0.41±0.09	0.32±0.07	0.48±0.08	0.26±0.05	0.42±0.08
HIP	GSH (nmol/mg protein)	1.20±0.50	2.17±0.87	1.72±0.26	1.61±0.18	2.27±0.77	2.15±0.95
	GSSG (nmol/mg protein)	2.45±1.18	2.53±0.86	3.14±0.46	1.63±0.29	3.80±1.52	3.19±1.48
	GSH/GSSG (redox status)	0.50±0.06	0.97±0.51	0.55±0.11	1.02±0.18	0.65±0.30	0.67±0.10
STR	GSH (nmol/mg protein)	1.99±0.13	1.79±0.17	1.81±0.17	1.96±0.34	1.80±0.14	1.76±0.12
	GSSG (nmol/mg protein)	2.30±0.20	1.77±0.45	1.67±0.20	1.66±0.17	1.48±0.29	1.89±0.34
	GSH/GSSG (redox status)	0.87±0.06	1.04±0.19	1.09±0.22	1.20±0.38	1.25±0.32	0.95±0.11

Table 1. GSH: reduced glutathione; GSSG: glutathione disulfide. Values are expressed as mean ± S.E.M. of 4-6 animals per group. *Significantly different of control ($p \leq 0.05$); two-way ANOVA followed by Bonferroni's test.

Figure 1. JPG

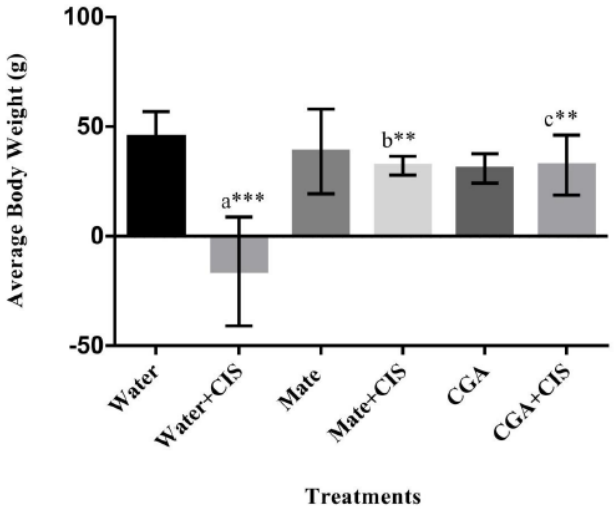


Figure 2.JPG

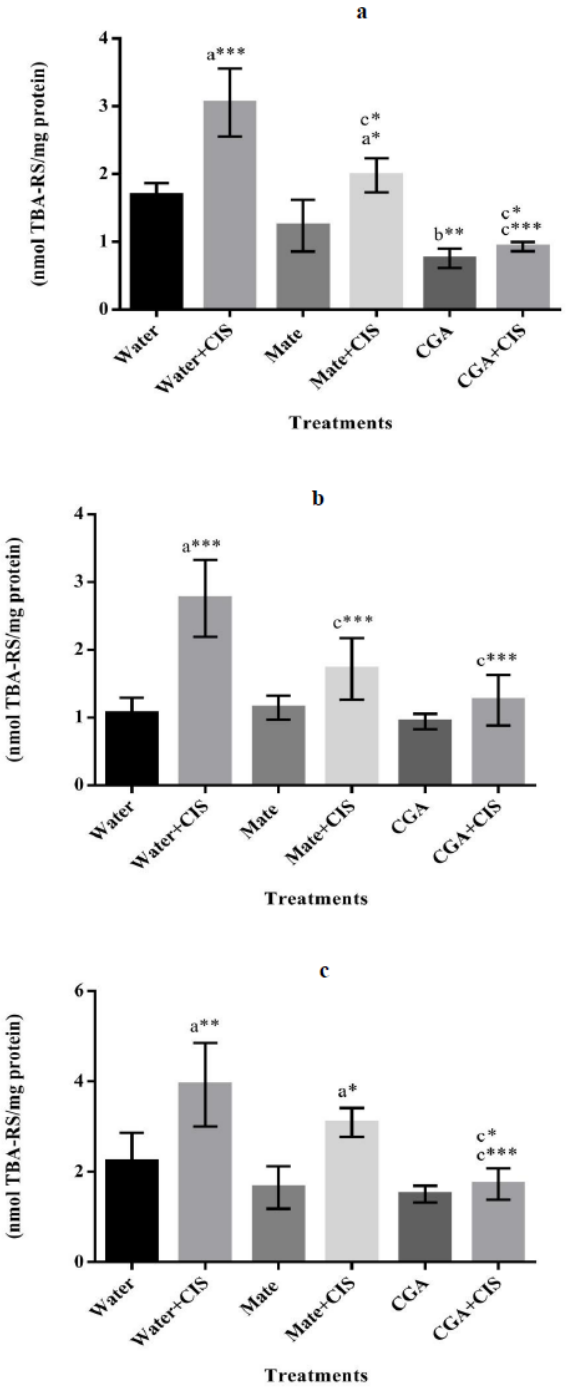


Figure 3.JPG

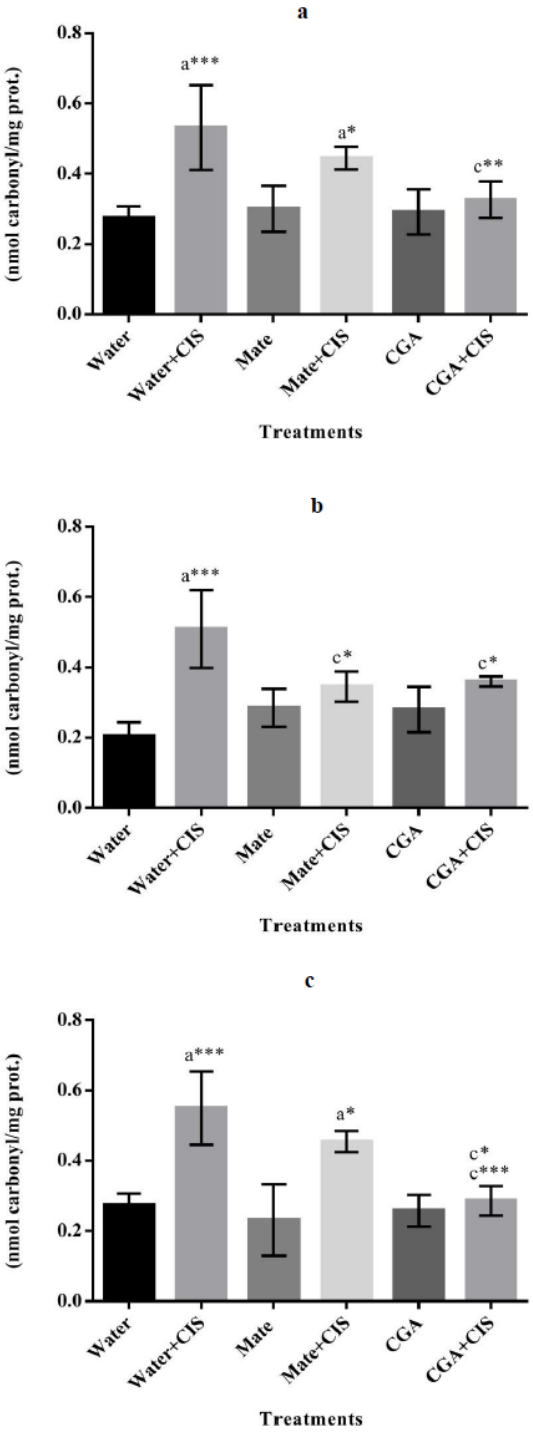


Figure captions

Fig. 1 Body weight changes in rats exposed to CIS (6 h per day for 21 days). Experimental groups designed: Water/Water+CIS (1,5 µl/g), Mate/Mate+CIS (1,5 µl/g), CGA/CGA+CIS (0,5 µl/g). Every recording was made 5 minutes before the immobilization and/or isolation of animals. Data represent the means ± SEM (n=4-6). Two-way ANOVA + Tukey's test; **a**=Water/Water+CIS; **b**=Water+CIS/Mate+CIS; **c**=Water+CIS/CGA+CIS. *p≤0.05, **p≤0.01, ***p≤0.001.

Fig. 2 Effect of Mate (1,5 µl/g) and CGA (0,5 µl/g) on CIS-induced levels of lipid peroxidation in the cortex (a), hippocampus (b) and striatum (c) of rats. Animals were subjected to immobilization (stress) or isolation (control) during 6 h for 21 days. At the end of treatments, the brain regions were collected. Results are presented as mean values ± S.E.M of n=4-6 rats per group, analyzed by two-way ANOVA followed by Tukey's test. **a**=Water/Water+CIS, Mate/Mate+CIS, CGA/CGA+CIS; **b**=Water/Mate, Water/CGA, CGA/Mate; **c**=Water+CIS/Mate+CIS, Water+CIS/CGA+CIS, CGA+CIS/Mate+CIS. *p≤0.05, **p≤0.01, ***p≤0.001.

Fig. 3 Effect of Mate (1,5 µl/g) and CGA (0,5 µl/g) on CIS-induced levels of protein carbonylation in the cortex (a), hippocampus (b) and striatum (c) of rats. Animals were subjected to immobilization (stress) or isolation (control) during 6 h for 21 days. At the end of treatments, the brain regions were collected. Data are presented as mean values ± S.E.M of n=4-6 rats per group, analyzed by two-way ANOVA followed by Tukey's test. **a**=Water/Water+CIS, Mate/Mate+CIS, CGA/CGA+CIS; **b**=Water/Mate, Water/CGA, CGA/Mate; **c**=Water+CIS/Mate+CIS, Water+CIS/CGA+CIS, CGA+CIS/Mate+CIS. *p≤0.05, **p≤0.01, ***p≤0.001.

4.3 Manuscrito 1

Original research

In vivo genotoxic and antigenotoxic activity of *Ilex paraguariensis* extracts

Ana C. Colpo[1*], Hemerson Rosa[1], Andréas S.L. Mendez[3], Mauricio Lehmann[2], Vanderlei Folmer[1]

[1]Programa de Pós-Graduação em Bioquímica, Universidade Federal do Pampa (UNIPAMPA), Uruguaiana-RS, Brazil.

[2]Programa de Pós-Graduação em Biologia Celular e Molecular Aplicada à Saúde (PPGBIOSAÚDE), Universidade Luterana do Brasil, ULBRA, Canoas-RS, Brazil.

[3]Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS), Avenida Ipiranga 2752, CEP 90610-000, Porto Alegre-RS, Brazil.

[*]Corresponding author: Ana C. Colpo. Universidade Federal do Pampa, UNIPAMPA, Campus Uruguaiana, BR 472 Km 592. CEP 97500-970- Caixa Postal 118, Uruguaiana-RS, Brazil. Electronic address: anaccolpo@hotmail.com

Abstract

Yerba mate, a product made with branches and leaves from *Ilex paraguariensis* is traditionally consumed as a typical drink in South America. The bioactive compounds in *yerba mate* extracts confer to the beverage several proven biological properties such as antioxidant activities, anti-inflammatory, neuroprotective, and so on. Considering that the large part of the population in south Brazil, Argentina and Uruguay consumes mate beverage, the objective of this study was to investigate the genotoxic and the antigenotoxic potentials of *yerba mate* extracts by the SMART (Somatic Mutation and Recombination Test) in *Drosophila melanogaster* wings. To evaluate the genotoxic activity, larvae obtained from standard (ST) and high bioactivation (HB) crosses were treated with crude extracts 1, 5 and 10**.** For the evaluation of antigenotoxic activity, the extracts were associated with two damage inducing agents: ethyl methanesulfonate (EMS) and mitomycin C (MMC) in co and post-treatment. The results obtained for genotoxic activity showed that *yerba mate* extracts did not induce an increasement in the frequency of spots, when compared to control groups**.** The results for the evaluation of antigenotoxicity indicated that the mate reduced the frequency of mutant spots in co-treatment, when compared to those treated with only the damage inducing agents. However, the extracts could present genotoxicity when administered after damage. Overall, the results indicate that the modulation by phytochemicals in xenobiotics defence actions, results in multiple outcomes, including beneficial health effects and undesirable consequences.

Keywords

Yerba mate, phenolic compounds, *Drosophila melanogaster*, SMART

1. Introduction

Yerba mate is a product manufactured with leaves and branches of the tree named *Ilex paraguariensis* (Bracesco et al., 2011). The consumption of *yerba mate* is traditional in south America border region, but its use as dietary supplements is increasing around the world due to its important antioxidant capacity (Bracesco et al., 2011; Colpo et al., 2016). Numerous studies have evaluated the association between *yerba mate* extracts consumption and the increase of antioxidant defenses in an organism.

However, effects as prevention of DNA damage (Barg et al., 2014) and capacity to decrease cancer cell proliferation (Amigo-Benavent et al., 2017), have also been reported. On the other hand, epidemiological studies have described positive association between *yerba mate* consumption and cancers of aerodigestive tract, esophagus, stomach, larynx, lung, cervix uteri, bladder, and kidney (Dasanayake et al., 2010; De Stefani et al., 2011).

Of note, in Argentina, Brazil and Uruguay the beverage is named “mate” or “chimarrão” and its traditional consumption occurs adding hot water to a gourd with the *yerba mate* (Bracesco et al., 2011). The water is drank through a metallic implement called “bombilla” and the process is repeated many times (Colpo et al., 2016; Amigo-Benavent et al., 2017).The hot water temperature (at about 80°C) and the presence of polycyclic aromatic hydrocarbons produced in the *yerba mate* manufacturing process has been associated to the carcinogenic effect already described (Islami et al., 2009, Kamangar et al., 2008). Nevertheless, there was little evidence to validate these endpoints.

Despite mate consumption occurs with sequential extractions, there are still few groups analyzing the extracts obtained by this form. Studies that observed the compounds in extracts obtained mimicking a legitimate one, showed that the major compound in mate is chlorogenic acid, but caffeine, theobromine and caffeic acid are also present in considerable concentrations (Meinhart et al., 2010, Colpo et al., 2016). Considering the polyphenols

compounds capacity to reduce oxidative damage (Halliwell and Gutteridge 2007) and its ability to modulate the activity of multiple targets involved in carcinogenesis (Fantini et al., 2015), investigations about its possible interactions with genetic material have stirred interest.

At present, there is consensus in exploring the interaction between nutrients and genes. The dietary components can be protective, carcinogenic, and mutagenic agents (Elsamanoudy et al., 2016). In this study, the Somatic Mutation and Recombination Test (SMART) of *Drosophila melanogaster* was used to determine the genotoxic effects of mate (*Ilex paraguariensis* extracts). SMART using wing spots in *D. melanogaster* is a *in vivo* assay that measures loss of heterozygosity (LOH) induced to detect mutational and mitotic recombinational events and quantifies the recombinagenic activity of chemicals (Andrade et al., 2004).

2. Materials and methods

2.1 Reagents and extracts preparation

In order to evaluate the genotoxic activity of *yerba mate* extracts, Urethane (URE, CAS Nº 51-79-6; Sigma-Aldrich Company, St. Louis, MO, USA) was used as positive control. The genotoxins Ethyl methanesulphonate (EMS, CAS no. 62-50-0; Sigma-Aldrich, St Louis, MO, USA) and Mitomycin C (MMC, CAS no. 50-07-7; Bristol-Myers Squibb, São Paulo, Brazil) were applied to access the antigenotoxic effects of the extracts.

Aqueous extracts were obtained by recreating the mate preparation process using the brand Baldo®, from Uruguay. Mate was prepared in a medium size gourd, with the *yerba mate* occupying two thirds of the bowl volume (85 g). The free volume was filled with water (70 mL) at 80^0C. The water was remained in contact with the *yerba mate* for one minute. The water was then removed through a pump attached to a suction system. Extracts were successively collected, and infusions numbers 1, 5 and 10 were stored for analysis. The extracts were then filtered using filter paper, thickness 205 µm (J.Prolab®, S.J.dos Pinhais,

Brazil), and stored in Eppendorf tubes. After extraction, the material was stored and kept in a freezer (-18^{0}C) until analysis (Colpo et al., 2016).

2.2 Total polyphenol content (TPC) quantification

The total polyphenol in *yerba mate* extracts was determined by UV–visible spectrophotometry using the Folin-Cioacalteau method with modifications (Singleton, Joseph, & Rossi, 1965). TPC was expressed in mg of Gallic acid equivalents (GAE) per mL of aqueous extract, which was analyzed using 0.05 mL extract.

2.3 Somatic Mutation and Recombination test (SMART)

By the use of *D. melanogaster* strains, which carry specific genetic markers (*mwh* and *flr*^{3}) on the left arm of chromosome 3 it is possible to detect different genetic end-points by the SMART assay (Graf et al., 1984). Because standard (ST) and high-bioactivation (HB) crosses express cytochrome P450 metabolization enzymes of distinct forms, the two crosses were used in the assessment of genotoxic potential of mate.

The standard cross (ST) use virgin females from the *flr*^{3}/In(3LR)*TM3*, ri p^p sep l(3)89Aa bx^{34e} e Bd^S strain, and the high bioactivation cross (HB) use virgin females from the ORR/ORR; *flr*^{3}/In(3LR)*TM*3, ri p^p sep l(3)89Aa bx^{34e} e BdS strain. These females, from both strains, were crossed with *mwh/mwh* males (Lindsley and Zimm, 1992). Then, they were left in the culture bottles containing a solid agar base (5% w/v agar-agar in water) covered with a 5-mm yeast supplemented with sucrose for 8 h to lay eggs. After 3 days, the larvae were collected and washed.

2.4 Genotoxic activity

Larvae of both crosses were transferred to flasks containing 1.5 g of *Drosophila* Instant Medium (Formula 4-24, Carolina Biological Supply, Burlington, NC) hydrated with the mates 1, 5 and 10. Water and urethane 20 mM were used as negative and positive control, respectively.

2.5 Antigenotoxic activity

The differences between EMS and MMC concentrations used in co and post-treatments are related to the time of exposure. In co-treatment was used a lower concentration of genotoxin, however the larvae were chronically exposed to treatments (which means 48 h).

2.5.1 Co-treatment

The 3-day-old larvae were fed on the medium containing 1.5 g of *Drosophila* Instant Medium and 5 mL of solutions test. The larvae were treated with genotoxin (EMS 5mM or MMC 0.05 mM) or with a combination of crude extracts (mates 1, 5 and 10) + genotoxin. In the negative control group was added water to the medium. All treated larvae were allowed to feed on this medium for the rest of their development, which corresponds to approximately 48 h (Van Schaik and Graf, 1993). All experiments were carried out at 25^0C and 65% relative humidity.

2.5.2 Post-treatment

For acute treatment, the 3-day-old larvae were distributed into Plexiglas tubes. The tube bottom end is coating with fine nylon gauze, allowing larvae to feed. Briefly, 50-mL beakers containing 0.3 g of powdered cellulose (Merck; Darmstadt, Germany) and 2mL of distilled water or genotoxin solution were placed in the tubes. The larvae were fed through the gauze on water or one concentration of the mutagen cellulose suspension for 3 h (EMS, 46 mM) or 6 h (MMC, 0.12 mM). After feeding, the larvae of both groups were washed and transferred to several vials containing 1.5 g of *Drosophila* Instant Medium with the addition of extracts 1, 5 and 10 or water.

2.6 Scoring of wings

Approximately 10 -12 days after egg laying, the emerging adult flies were collected and stored in 70% ethanol. The wings were mounted in Faure's solution (gum arabic, 30 g; glycerol,20 mL; chloral hydrate, 50 g; water, 50 mL). For recording the two different

categories of wing spots, both the dorsal and the ventral surfaces of the male and female wings were analyzed microscopically at 400x magnification (Graf et al., 1984).

Both ST and HB crosses produce two types of progeny that are distinguished phenotypically based on the Bd^s marker: trans-heterozygous flies (*mwh/flr*^{3}) for the recessive wing cell markers multiple wing hair (*mwh*) and flare (*flr*^{3}). Induced loss of heterozygosity in the marker-heterozygous genotype leads to two types of mutant clones: single spots, either *mw*h or *flr*^{3}, which result from point mutation, chromosome aberration, and/or somatic recombination, and twin spots, consisting of both *mwh* and *flr*^{3} subclones, which originate exclusively from somatic recombination (Graf et al., 1984).

2.7 Statistical analysis

To determine the statistical differences in the polyphenol content in mates the data were submitted for analysis of variance (ANOVA) one way. Data were expressed as the means ± standard deviations (SD), and differences between the groups were considered significant when $p \leq 0.05$. To compare the means of TPC in extracts and the groups of SMART, the Bonferroni's test was used.

To describe the results of SMART the multiple-decision procedure are applied, which enables four different diagnoses: positive, weakly positive, negative, or inconclusive (Frei and Würgler, 1988). Two alternative hypotheses are used to distinguish among the possibilities of a positive, inconclusive, or negative result in *Drosophila* mutagenicity tests (Frei and Würgler, 1988).

In the null hypothesis one assumes that there is no difference in the mutation frequency between control and treated series. The alternative hypothesis postulates that the treatment results in an increased mutation frequency that is ***m*** times the spontaneous frequency. If neither of the 2 hypotheses is rejected, the results are considered inconclusive. If the two hypotheses are rejected, the result is considered weak positive (Frei and Würgler,

1988). Results were analyzed with the non-parametric U test of Mann, Whitney and Wilcoxon (Frei and Würgler, 1988). The frequencies of each type of mutant clone per fly of a treated series were compared pairwise using the conditional binomial test of Kastenbaum and Bowman (1970).

3. Results and discussion

"Mate" is especially rich in polyphenols and its antioxidant potential has been related to beneficial effects in living organisms. However, there is a gap in understanding about its mutagenic and antimutagenic action. To improve this knowledge, the present study investigated its biological activities employing the SMART in *D. melanogaster* by using extracts produced mimetizing the legitimate one.

3.1. Total polyphenol content (TPC)

The highest concentration of total polyphenols was found in mate 5 (mate 5> mate 1 > mate 10). The **Table 1** shows these results, were it is possible to note that the TPC was significantly lower in mate 10. The decrease in compounds levels observed here is justified by the trend to lower its content with multiple extractions.

This observation is in accordance to a study previously reported by our group (Colpo et al., 2016). The concentration of phenolic compounds in mates is not related to the mutagenic events, but may have some association with antimutagenesis in post-treatment, which leads us to consider that the content of compounds in very diluted extracts is sufficient to give it ability to interfere in modulatory pathways of various functions.

Of note, Lima et al (2014) demonstrated that the most concentrated and the most diluted extracts of *Ilex paraguariensis* presented a similar effect in survival rate of *C. elegans*. Colpo et al (2016) observed that the antioxidant activity is significant in extracts very diluted. To complete, recently our group observed that the capacity to reduce the lipid

peroxidation and its self-propagating in fruit flies is more effective in extracts more diluted (data no show).

3.2 Genotoxic and antigenotoxic activity

SMART test in ST and HB crosses of *D. melanogaster* allows us to introduce the genotoxic potential of *yerba mate* extracts (**Table 2**). *Yerba mate* extracts did not affect significantly the occurrence of clone spot in marker trans-heterozygous (*mwh/flr*^{3}) adult flies, when compared with the negative controls of both crosses. All extracts tested produced negative or inconclusive results in all spot categories considered. On the basis of these findings it is possible to assume that the extracts tested lack mutagenic and recombinagenic action in the SMART in both crosses.

It is important to observe that the strains of the high bioactivation (HB) exhibits a high constitutive level of cytochrome P450 and high bioactivation capacity, being particularly sensitive to pro-carcinogens and pro-mutagens (Graf and Schaik, 1992). Our experiment using urethane, an indirect action genotoxic agent as positive control, increased in 545.91% the frequency of total spots in HB cross. The extracts, in turn, do not presented association with the metabolizing enzymes constitutive levels in fruits flies. Similar data were reported by Silva et al. (2013) and Rodrigues et al. (2017).

To observe the antigenotoxic potential of *yerba mate* extracts the genetic damages were induced by EMS and MMC in ST cross. These mutagenic agents were used as damage inducers in co and post-treatment.

The results showed that in EMS and MMC co-treatment all extracts tested showed weakly positive results (**Table 3**). The weakly positive association observed would mean that the mutation frequency is significantly lower than the control frequency. Many studies have shown that polyphenols confer antimutagenic properties to natural products. Honey demonstrated strong antimutagenicity, and phenolic compounds were found to be one of the

important factors contributing to this effect (Saxena et al., 2012). Apple extracts showed capacity to modulate detoxification related to genes spotted on the custom array that are related to important functions such as, tumor suppression, cell cycle control, cell signaling as well as apoptosis (Veeriah et al., 2008). Due to strong antioxidant property, black tea inhibits the development of various cancers by regulating oxidative damage of biomolecules, endogenous antioxidants, and pathways of mutagen and transcription of antioxidant gene pool (Singh et al., 2017).

The results observed here can be, at least in part, associated to the capacity of polyphenols on modulating expression of selected genes related to toxicological defence and/or induction of repair enzymes which reduce the action of the mutagenic agents MMC and EMS. Moreover, additional studies are necessary to better evaluate the possible antigenotoxic activity of mate infusion.

Regarded to tests in acute post-treatment approach, when the larvae were exposed to extracts for 3 hours it was possible to observe that they do not have mutagenic activity *per se* (data no shown). Moreover, data from EMS induction demonstrated that the extracts do not produce antimutagenic effects. It is possible to observe that the null hypothesis can be ruled out in all treatments, which indicates that when the compounds from extracts act in association with EMS they can produce mutation, mitotic recombination or both. The **Table 4** shows these evidences.

To check the antimutagenic effects of extracts in chronic post-treatment, at first the *mwh/flr*^{3} flies were exposed to extracts 1, 5 and 10 for 6 hours. These conditions do not originate alterations in the frequency of spots and in the total spots; the diagnosis was negative or inconclusive in all tests (data no shown). When the progenies were exposed to MMC, the results showed that extract 1 produces reduction in the frequency of twin spots, and extract 5 in the frequency of small single spots. Despite this, the total spot frequency was

not reduced, suggesting that *yerba mate* not have antigenotoxic effect in chronic treatment, too (**Table 5**).

The **Tables 4** and **5** show that *yerba mate* extracts produced an increase in the number of total spots in all treatments. In general the results were positive or weak positive demonstrating that the extracts could present genotoxicity when administered after damage. These effects may be due to the capacity of natural polyphenols on altering the activity of CYP, either via direct interactions with the enzymes or by affecting CYP gene expression (Korobkova, 2015).

Inhibitory effects involve the formation of a covalent bond between the polyphenol and the CYP3A4 (enzyme from cytochrome P450 (CYPs) analogous to the CYP6 enzyme family in *Drosophila*), which leads to the inactivation of the enzyme (Dresser, Spence and Bailey, 2000; Tijet et al., 2001; Basheer and Kerem, 2015). The administration of flavonoids and clinically used drugs may cause flavonoid-drug interactions by modulating the pharmacokinetics of certain drugs, which results in an increase in their toxicity or a decline in their therapeutic effect, depending on the structure (Tang and Steams, 2001; Kyselova, 2011). To complete, there are reports indicating that bioactive components of fruits and vegetables can interfere with the expression of DNA repair enzymes (Ferguson et al., 2015).

In SMART artepillin, a phenolic acid from honey can elicit mutagenicity dose-dependent. It was attributed to its pro-oxidant activity, which manifests as ROS production (Rodrigues et al., 2017). *Vernonanthura polyanthes* aqueous extracts from its leaves in association with doxorubicin, a potent clastogenic agent of direct action, produce increase in its mutagenic potential, increasing the number of mutations in *D. melanogaster* somatic cells (Guerra-Santos et al., 2016).

To complete, a modulatory activity against damage induced previously by MMC was observed in mate 1 as regard to reduction in recombinatory event. In mate 5, the reduction in

the frequency in small single spots could be result from a metabolic inactivation of the tested compound, so that cells undergoing cell division in pupation are no susceptible at risk for genotoxic effects (Frei and Würgler, 1988). The recombination is maintained nonetheless. SMART in *mwh/TM3* flies crosses can confirm this tendency. Using the wings heterozygous for multiple inversions on the *TM3* balancer chromosome, it is possible to separate mutational events from recombinational events because the recombinational events are eliminated in flies with this genotype (Castro et al., 2008). As regard to this result, it is necessary to consider the important antioxidant capacity of extracts; this effect could be responsible for the activation of underlying antimutagenic mechanisms that are able to improve detoxification.

4. Conclusion

This research promotes a progress in understanding the genotoxic and antigenotoxic effects of *yerba mate* based beverages. The results indicate that the mate infusion lack mutagenic and recombinagenic action in somatic cells of *D. melanogaster*. However, the data allow us to hypothesize that the action of extracts is based on distinct mechanisms in antimutagenic approach, which are related with the DNA repair mechanism and/or interaction with CYP activity in a drug and xenobiotic metabolism process. To complete, it was observed that similar effects are produced by extracts that have higher or lower TPC levels.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgement

This work was supported by CAPES, UNIPAMPA, URCAMP and ULBRA.

References

Amigo-Benavent, M., Wang, S., Mateos R., et al. (2017). Antiproliferative and cytotoxic effects of green coffee and yerba mate extracts, their main hydroxycinnamic acids, methylxanthine and metabolites in different human cell lines. Food and Chemical Toxicology. doi.org/10.1016/j.fct.2017.05.019.

Andrade, H.H.R., Reguly, M.L., Lehmann, M. (2004). Wing somatic mutation and recombination test. D.S. Henderson (Ed.), *Drosophila* Cytogenetics Protocols, Humana Press, New Jersey.

Barg, M., Rezin, G.T., Leffa, D. D., et al. (2014). Evaluation of the protective effect of *Ilex paraguariensis* and *Camellia sinensis* extracts on the prevention of oxidative damage caused by ultraviolet radiation. Environmental Toxicology and Pharmacology. doi: 10.1016/j.etap.2013.11.028.

Basheer, L. and Kerem Z. (2015). Interactions between CYP3A4 and Dietary Polyphenols. Oxid Med Cell Longev. doi.org/10.1155/2015/854015.

Bracesco, N., Sanchez, A.G., Contreras, V.et al. (2011). Recent advances on *Ilex paraguariensis* research: Minireview. Journal of Ethnopharmacology. doi:10.1016/j.jep.2010.06.032.

Castro, A.J.S., Grisolia, C.K., de Araújo B.C., et al. (2008). Recombinogenic effects of the aqueous extract of pulp from pequi fruit (*Caryocar brasiliense*) on somatic cells of *Drosophila melanogaster*. Genet. Mol. Res. 7(4):1375-83.

Colpo, A.C., Rosa H., Lima M.E., et al. (2016). Yerba mate (*Ilex paraguariensis* St. Hill.)-based beverages: How successive extraction influences the extract composition and its capacity to chelate iron and scavenge free radicals. Food Chemistry. doi:10.1016/j.foodchem.2016.04.059.

Dasanayake A.P., Silverman A.J., Warnakulasuriya S. (2010). Mate drinking and oral and oro-pharyngeal cancer: a systematic review and meta-analysis. Oral Oncol. 46:82–86.

De Stefani, E., Moore, M., Aune D. et al. (2011). Mate consumption and risk of cancer: a multi-site case-control study in Uruguay. Asian Pac. J. Cancer. Prev. doi.org/10.1016/j.etap.2013.11.028.

Dresser, G.K., Spence, J.D., Bailey, D.G. (2000). Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. Clin Pharmacokinet. 38(1):41-57.

Elsamanoudya, A.Z., Neamat-Allah M.A.M., Mohammad F.A.H. et al. (2016). The role of nutrition related genes and nutrigenetics in understanding the pathogenesis of cancer. Journal of Microscopy and Ultrastructure, 4: 115–122.

Fantini, M., Benvenuto, M., Masuelli, L., et al. (2015). *In Vitro* and *in Vivo* Antitumoral Effects of Combinations of Polyphenols, or Polyphenols and Anticancer Drugs: Perspectives on Cancer Treatment. Int J Mol Sci. doi: 10.3390/ijms16059236.

Ferguson, L.R., Chen, H., Collins, A.R. (2015). Genomic instability in human cancer: Molecular insights and opportunities for therapeutic attack and prevention through diet and nutrition. Seminars in Cancer Biology, doi.org/10.1016/j.semcancer.2015.03.005.

Frei, H., Würgler, F.E. (1988). Statistical methods to decide whether mutagenicity test data from *Drosophila* assays indicate a positive, negative or inconclusive result. Mutat. Res. 203: 297-308.

Frei, H., Würgler, F.E. (1996). Induction of somatic mutation and recombination by four inhibitors of eukaryotic topoisomerases assayed in the wing spot test of *Drosoph. Melanogaster*. Mutagen. 11: 315-325.

Graf, U., Van Schaik, N. (1992). Improved high bioactivation cross for the wing somatic mutation and recombination test in *Drosophila melanogaster*. Mutat Res., 271(1):59-67.

Graf, U., Würgler, F.E., Katz, A.J., et al. (1984). Somatic mutation and recombination test in *Drosophila melanogaster*. Environ. Mol. Mutagen. 6: 153-188.

Guerra-Santos, I.J., Rocha, J.D., Vale, C.R. (2016). *Vernonanthura polyanthes* leaves aqueous extract enhances doxorubicin genotoxicity in somatic cells of Drosophila melanogaster and presents no antifungal activity against Candida spp. Brazilian Journal of Biology, doi.org/10.1590/1519-6984.04615.

Halliwell, B., & Gutteride, J. M. C. (2007). Free radicals in biology and medicine (4th ed.). New York: Oxford University Press Inc.

Islami, F., Boffetta, P., Ren, J., et al. (2010). High-temperature beverages and Foods and Esophageal Cancer Risk - A Systematic Review. Int J Cancer. doi: 10.1002/ijc.24445.

Kamangar, F., Schantz, M.M., Abnet C.C., et al. (2008). High Levels of Carcinogenic Polycyclic Aromatic Hydrocarbons in Mate Drinks. Cancer Epidemiol Biomarkers Prev. doi:10.1158/1055-9965.EPI-08-0025.

Kastenbaum, M.A., Bowman, K.O. (1970). Tables for determining the statistical significance of mutation frequencies. Mutat. Res. 9: 527- 549.

Korobkova, E.A. (2015). Effect of Natural Polyphenols on CYP Metabolism: Implications for Diseases. Chem Res Toxicol. doi: 10.1021/acs.chemrestox.5b00121.

Kyselova, Z. (2011). Toxicological aspects of the use of phenolic compounds in disease prevention. Interdiscip Toxicol. doi: 10.2478/v10102-011-0027-5

Lima M.E., Colpo A.C., Salgueiro W.G.et al. (2014). *Ilex paraguariensis* extract increases lifespan and protects against the toxic effects caused by paraquat in *Caenorhabditis elegans*. Int J Environ Res Public Health. doi: 478 10.3390/ijerph111010091.

Lindsley, D.L., Zimm, G.G. (1992). The Genome of *Drosophila melanogaster*. Academic Press Inc., San Diego.

Meinhart, A. D., Bizzotto, C. S., Ballus, C. A., et al. (2010). Methylxanthines and phenolics content extracted during the consumption of mate (*Ilex paraguariensis* St. Hil) beverages. J Agric Food Chem. doi: 10.1021/jf903781w.

Rodrigues, C.R.F., Plentz, L.C., Flores, M.A., et al., (2017). Assessment of genotoxic and antigenotoxic activities of artepillin C in somatic cells of *Drosophila melanogaster*. Food Chem. Toxicol. doi.org/10.1016/j.fct.2017.01.005.

Saxena, S., Gautam, S., Maru, G., et al. (2012). Suppression of error prone pathway is responsible for antimutagenic activity of honey. Food Chem. Toxicol. 50: 625- 633.

Silva, R.A., Dihl, R.R., Nascimento, D. et al. (2013). Evaluation of antioxidant and mutagenic activities of honey-sweetened cashew apple nectar. Food Chem Toxicol. doi: 10.1016/j.fct.2013.08.026.

Singh, B.N., Rawat, A.K., Bhagat, R.M., Singh, B.R. (2017). Black tea: Phytochemicals, cancer chemoprevention, and clinical studies. Crit Rev Food Sci Nutr. doi: 10.1080/10408398.2014.994700.

Singleton, V. L., Joseph, A., & Rossi, J. (1965). Colourimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16, 144–149.

Tang, W. and Stearns, R.A. (2001). Heterotropic cooperativity of cytochrome P450 3A4 and potential drug-drug interactions. Curr Drug Metab. 2(2):185-98.

Tijet, N., Helvig, C., Feyereisen, R. (2001). The cytochrome P450 gene superfamily in Drosophila melanogaster: annotation, intron–exon organization and phylogeny. Gene, 262:189-198.

Veeriah, S., Miene, C., Habermann, N. (2008). Apple polyphenols modulate expression of selected genes related to toxicological defence and stress response in human colon adenoma cells. Int.J.Cancer, 122:2647-2655.

Tables

Table 1- Quantification of the Total polyphenol content (TPC) in extracts.

Extracts	**1**	**5**	**10**
TPC (GAE/mL)	1.033 ±0.05	1.233 ±0.13	0.547 ±0.24[a*b**]

a= statistically significant differences between the mate 1 and others.
b= statistically significant differences between the mate 5 and others**.**
Each value is presented as mean ± SD (n = 3).
* Indicated decrease in TPC, when $p \leq 0.05$.
* ($p \leq 0.05$) ** ($p \leq 0.01$) *** ($p \leq 0.001$)
The TPC was expressed in terms of GAE per mL of aqueous extract.

Table 2- ST and HB crosses after exposure of third instar larvae to different extracts (mates). Results of SMART in *mwh/flr*^{3} progeny.

Crosses/ genotypes	Number of flies (N)	Spots per fly (no. of spots)/statistical diagnosis [a]				Spots with *mwh* clone [c]
		Small single spots [b] (1-2 cells) (*m=2*)	Large single spots [b] (>2 cells) (*m=5*)	Twin spots (*m = 5*)	Total spots [b] (*m = 2*)	
ST cross *mwh/ flr*^{3}						
NC	20	0.65(13)	0.15(03)	0.00(00)	0.80(16)	16
PC	20	3.90(78)+	0.30(06)i	0.70(14)+	4.90(98)+	98
Extract 1	20	0.70(14)i	0.15(03)i	0.15(03)i	1.00(20)i	20
Extract 5	20	0.50(10)-	0.15(03)i	0.15(03)i	0.80(16)-	16
Extract 10	20	0.55(11)-	0.10(02)i	0.00(00)i	0.65(13)-	13
HB cross *mwh/ flr*^{3}						
NC	20	1.20(24)	0.15(03)	0.10(02)	1.45(29)	29
PC	20	23.40(468)+	5.55(111)+	3.00(60)+	31.95(639)+	633
Extract 1	20	1.00(20)-	0.05(01)i	0.10(02)i	1.15(23)-	23
Extract 5	20	1.05(21)-	0.25(05)i	0.15(03)i	1.45(29)-	29
Extract 10	20	1.00(20)-	0.15(03)i	0.00(00)i	1.15(23)-	23

[a] Statistical diagnoses according to Frei and Würgler (1988): U test of Mann, Whitney and Wilcoxon: **(+)** positive;**(-)** negative; **(i**) inconclusive. $p \leq 0.05$, vs negative control (NC).
[b] Including rare *flr*^{3} single spots.
[c] Considering *mw*h clones from *mwh* single and twin spots.
[d] NC: negative control: water.
[e] PC: positive control: urethane 20 mM.

Table 3- ST cross after chronic exposure of 3rd instar larvae to co-treatment of extracts (mates) with EMS and MMC. Results of SMART in *mwh/flr*^{3} offspring.

Treatments		Number of flies (N)	Spots per fly (no. of spots)/statistical diagnosis [a]				Spots with *mwh* clone [c]
			Small single spots [b] (1-2 cells) (*m=2*)	Large single spots [b] (>2 cells) (*m=5*)	Twin spots (*m = 5*)	Total spots [b] (*m = 2*)	
NC[d]		40	0.58(23)	0,20(08)	0.00(00)	0.75(30)	30
MMC (mM)	Water/ Extract						
0.05	Water	40	22.85(914)*	27.05(1082)*	12.88(515)*	62.78(2511)*	2417
0.05	1	40	14.10(564)w+	18.23(729)w+	9.70(388)w+	42.03(1681)w+	1605
0.05	5	40	14.95(598)w+	17.25(690)w+	8.35(334)w+	40.55(1622)w+	1558
0.05	10	40	15.05(601)w+	23.70(948)w+	12.58(503)+	51.30(2052)+	1943
EMS (mM)	Water/ Extract						
5	Water	40	34.90(1396)*	14.98(599)*	10.55(422)*	60.43(2417)*	2283
5	1	40	19.05(762)w+	12.95(518)w+	12.20(488)w+	44.20(1768)w+	1712
5	5	40	25.93(1037)w+	13.60(544)w+	13.78(551)w+	53.30(2131)w+	2043
5	10	40	32.35(1294)w+	17.80(712)w+	13.38(535)w+	63.53(2541)w+	2225

[a]Statistical diagnoses according to Frei and Würgler (1988) for comparison with corresponding control: **(+)** positive; **(-)** negative; (**i)** inconclusive and (**w+)** weakly positive (p = 0.05); p≤0.05, vs MMC 0.05 mM or EMS 5 mM.
[b] Including rare *flr*^{3} single spots.
[c] Considering *mwh* clones from *mwh* single and twin spots.
[d] NC: negative control, water.

Table 4- ST cross after acute exposure of 3rd instar larvae to post-treatment of extracts (mates) with EMS (3h). Results of SMART in *mwh/flr*^{3} offspring.

Treatments		Number of flies (N)	Spots per fly (no. of spots)/statistical diagnosis [a]				Spots with *mwh* clone [c]
			Small single spots [b] (1-2 cells) (*m=2*)	Large single spots [b] (>2 cells) (*m=5*)	Twin spots (*m = 5*)	Total spots [b] (*m = 2*)	
NC[d]		40	0.53(21)	0.10(04)	0.03(01)	0.63(25)	
EMS (mM)	Water/ Extract						
46mM	Water	40	7.33(293)+	8.30(332)+	6.95(278)+	22,58(903)+	789
46mM	1	40	10.38(415)w+	14.70(588)w+	15.45(618)+	40.45(1621)w+	1518
46mM	5	40	14.43(577)+	16.25(650)+	17.13(685)+	47.80(1912)+	1723
46mM	10	40	10.10(404)w+	11.05422)w+	13.53(541)+	34.68(1387)w+	1227

[a]Statistical diagnoses according to Frei and Würgler (1988, 1995) for comparison with corresponding control: **(+)** positive; (-) negative; (**i)** inconclusive and (**w+)** weakly positive (p = 0.05); p≤0.05, vs MMC 0.05 mM or EMS 5 mM.
[b] Including rare *flr*^{3} single spots.
[c] Considering *mwh* clones from *mwh* single and twin spots.
[d] NC: negative control, water.

Table 5- ST cross after chronic exposure of 3rd instar larvae to post-treatment of extracts (mates) with MMC (6h). Results of SMART in *mwh/flr*^{3} offspring.

Treatments		Number of flies (N)	Spots per fly (no. of spots)/statistical diagnosis [a]				Spots with *mwh* clone [c]
			Small single spots [b] (1-2 cells) (*m=2*)	Large single spots [b] (>2 cells) (*m=5*)	Twin spots (*m = 5*)	Total spots [b] (*m = 2*)	
NC[d]		20	0.60(12)	0.15(03)	0.10(02)	0.75(15)	
MMC (mM)	Water/ Extract						
0.12	Water	20	1.75(35)+	11.55(231)+	5.20(104)+	18.50(370)+	335
0.12	1	20	10.95(219)+	15.60(312)w+	4.95(99)-	31.50(630)w+	609
0.12	5	20	3.85(77)-	16.45(329)w+	9.75(195)+	30.05(601)w+	563
0.12	10	20	6.40(128)+	17.30(346)w+	7.60(152)w+	31.30(626)w+	577

[a]Statistical diagnoses according to Frei and Würgler (1988) for comparison with corresponding control: **(+)** positive; **(-)** negative; **(i)** inconclusive and **(w+)** weakly positive (p = 0.05); p≤0.05, vs MMC 0.05 mM or EMS 5 mM.

[b] Including rare *flr*^{3} single spots.

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

[d] NC: negative control, water.

5 DISCUSSÃO E CONCLUSÕES

Efeitos biológicos dos extratos de erva-mate foram o foco principal na projeção desta tese. O **artigo 1** e o **manuscrito 1** utilizaram *Drosophila melanogaster* como modelo. O principal objetivo do primeiro foi explorar a capacidade dos extratos reduzirem efeitos negativos associados ao consumo de dietas ricas em colesterol. O mesmo encontra-se aceito para publicação no **Brazilian Journal of Medical and Biological Research**. A conclusão principal deste foi que extratos de erva-mate são capazes de aumentar a resistência ao estresse e prolongar o tempo de vida de moscas da fruta.

O **artigo 2** trata de descrever os efeitos antioxidantes da infusão de mate e do CGA em um modelo de estresse crônico, neste os protocolos experimentais envolveram ratos wistar com a utilização de três regiões cerebrais: córtex, hipocampo e corpo estriado. O referido trabalho está publicado na revista **Applied Physiology, Nutrition and Metabolism.** Como principal desfecho observou-se que os dois tratamentos são hábeis para reduzir efeitos indesejáveis provocados por estresse oxidativo a nível destas estruturas cerebrais.

No **manuscrito 1** estão apresentados resultados sobre eventos genotóxicos causados por mutagênese, além disso avaliou-se a possibilidade do extrato ser indutor de dano. Propõe-se submeter estes resultados na revista **Food and Chemical Toxicology**. Resumidamente os dados informam que mate não induz eventos mutagênicos em mosca da fruta, no entanto atuando em associação com mutágenos pode aumentar sua toxidade.

O estresse oxidativo tem sido implicado na fisiopatologia do envelhecimento e de doenças associadas à idade (OTANI, 2013). Em referência ao **artigo 1** os dados demonstram que as propriedades antioxidantes da erva-mate aumentam a tolerância ao estresse, melhoram a coordenação da degradação, absorção e tráfego do colesterol e possuem ação na recuperação de funções de detoxificação, esses efeitos repercutiram em aumento do tempo de vida das moscas. Além disso, observou-se que a peroxidação lipídica e a carbonilação de proteínas, podem ser limitadas pelos extratos de erva-mate. Para completar, foi possível detectar que as cinco principais matesaponinas, compostos fenólicos e metilxantinas, previamente descritos na literatura, são preservados em sucessivas extrações.

Apesar destas observações, que revelam propriedades benéficas das bebidas a base de erva-mate, é importante ressaltar que o metabolismo energético nas moscas está associado a mecanismos complexos em que a dieta pode afetar positiva ou negativamente os eventos envolvidos nessas interações. Acredita-se que o uso de cepas mutantes para enzimas antioxidantes (PENG et al., 2009) e para DHR96 (HORNER et al., 2009) e o uso de moscas

fêmeas melhorariam estas conclusões e complementariam dados relacionados à influência hormonal associada ao gênero. Além disso, aspectos como quais matesaponinas são prevalentes em extrações sequências e a ação de compostos isolados nos parâmetros testados, são possibilidades de avanços neste campo.

Em relação aos efeitos dos extratos de erva-mate e seu composto majoritário, o CGA, em nível de SNC (**artigo 2**) estudos prévios revelaram que o estresse induzido por restrição produz alterações psicológicas e físicas, provocando dano oxidativo em diferentes regiões do cérebro, incluindo o hipocampo, o córtex e o estriado (LIU et al., 1996; COLÍN-GONZÁLEZ et al., 2015). Estresse crônico pode induzir uma grande produção de radicais livres, ao aumento de parâmetros oxidativos (malonaldeído (MDA), por exemplo), e a diminuição dos níveis de enzimas antioxidantes, incluindo GSH, SOD, GPx e CAT (SAMARGHANDIAN et al., 2017).

Os dados aqui apresentados confirmam que o dano oxidativo pode se manifestar com aumento da peroxidação lipídica e com danos a proteínas (HALLIWELL, 2001), o que também foi visto no estudo com *Drosophila*. Os animais submetidos a restrição de movimentos apresentaram aumento dos níveis de TBARS e de carbonilação de proteínas em todas as regiões testadas. Em contrapartida mate e CGA foram hábeis em reduzir significativamente esses níveis.

Ao relacionar os dados de todos os grupos submetidos à restrição verificou-se que no CTX e HIP, a peroxidação lipídica foi significativamente menor nos grupos tratados com mate e CGA. No STR, apenas CGA apresentou resposta significativa contra o estresse. Em relação à carbonilação de proteínas observou-se que todos os grupos tratados com CGA exibiram níveis mais baixos de carbonilação de proteínas em relação aos controles. Mate diminuiu significativamente a oxidação de proteínas apenas no hipocampo.

Comparando-se CGA com o extrato bruto evidencia-se que o composto isolado foi mais efetivo no controle do balanço redox no SNC. O que pode ser devido à ação sinérgica de outros compostos presentes no extrato bruto, que poderiam influenciar a biodisponibilidade de algumas moléculas. Em relação às regiões analisadas nota-se, ainda, que as células do hipocampo apresentam melhores respostas, o que se sugere que seja devido às características intrínsecas das células desta região, hipótese que deve ser futuramente investigada.

Para completar estresse por restrição produziu um aumento nos níveis de GSH somente em CTX, essa mudança pode ser interpretada como uma resposta adaptativa/compensatória ao dano oxidativo em curso. A mesma análise pode ser adotada para

a tendência observada nos grupos submetidos à restrição e tratados com mate ou CGA apresentarem aumento dos níveis GSH/GSSG nas três regiões estudadas.

No **manuscrito 1,** o SMART da asa de *Drosophila melanogaster* forneceu informações sobre o potencial dos extratos brutos de erva-mate induzirem perda de heterozigose resultantes de mutações genéticas, rearranjos cromossômicos, quebras ou perdas cromossômicas. A aplicação do teste, nos diferentes cruzamentos (padrão e aprimorado) resultou na observação de que os extratos, obtidos mimetizando a forma tradicional de consumo e com variáveis concentrações de compostos fenólicos, não são indutores de danos, independente do nível constitutivo de enzimas de metabolização do CYP no inseto. No entanto, mate pode agir potencializando danos produzidos por agentes mutagênicos quando aplicado após indução.

Compostos polifenólicos podem interagir com enzimas do CYP, especialmente com a CYP3A4, alterando sua expressão e atividade, o que é particularmente importante devido à implicação destas associações no metabolismo de drogas (BASHEER E KEREM, 2015). A expressão de enzimas do CYP é regulada por múltiplos processos incluindo modificações conformacionais em fatores de transcrição, sua translocação para o núcleo e sua ligação aos elementos de resposta na região promotora do gene. Os polifenóis naturais demonstraram influenciar a expressão enzimática afetando todos esses processos (KOROBKOVA, 2015). Neste contexto, as interações CYP-polifenóis explicam, pelo menos em parte, o aumento do número de manchas nos pós-tratamentos utilizando MMC e EMC como indutores de danos.

Na indução com EMS houve aumento do número de manchas simples pequenas, simples grandes e gêmeas, o que denota que estas foram induzidas em todas as fases da metamorfose e por diferentes processos de perda da heterozigosidade. Manchas pequenas normalmente se desenvolvem na fase de pupa e na maioria das vezes são decorrentes de aberrações cromossômicas, manchas grandes ocorrem durante o estágio larval e podem estar associadas a todos os tipos de alterações. O aumento das manchas gêmeas (*mwh* e *flr*^{3}), por sua vez, demostra que os eventos recombinatórios foram amplificados (FREI e WÜRGLER, 1988).

MMC também produziu um aumento na frequência do total de manchas, no entanto houve redução do número de manchas simples pequenas no mate 5 e de manchas gêmeas no mate 1, supondo-se que em algum momento pode haver a ação de uma atividade moduladora subjacente, onde pode estar incluída a eliminação de radicais livres e o aprimoramento da proteção antioxidante (ARUL e SUBRAMANIAN, 2013), um dos atributos dos polifenóis que deve ser considerado.

Tomados em conjunto os resultados aqui apresentados evocam que extratos de erva-mate podem atuar contra danos induzidos modulando diferentes vias. Compostos presentes em extratos de erva-mate podem desenvolver seus mecanismos agindo como sequestradores de radicais livres ou atuando vias de metabolização, ativação enzimática ou em processos celulares de reparo. Pode-se concluir que danos oxidativos podem ser contrabalanceados pelos compostos presentes no extrato de erva-mate, isto em um organismo inteiro ou em regiões isoladas, especialmente sensíveis ao metabolismo redox, como o cérebro.

Além disso, em relação a processos de detoxificação a ação dos extratos parece se relacionar à natureza química e a forma de metabolização do agente indutor. Por exemplo, observou-se que o extrato foi capaz de recuperar a ação da enzima GST quando esta teve redução de sua ação provocada pela composição da dieta. Em contra partida, quando se induziu danos através de genotoxinas o que se observou foi aumento dos eventos mutacionais associados, possivelmente por uma interação polifenol-CYP que reduziu a capacidade de metabolização celular e aumentou a toxidade da droga. Dados da literatura tem enfatizado essa relação, no entanto mais estudos são necessários para elucidá-la completamente. Esta é mais uma perspectiva que emerge dos resultados aqui apresentados.

Para completar, esses dados corroboram com outras investigações que mostram que o equilíbrio redox está envolvido na prevenção da gênese de muitas doenças crônicas. Os desequilíbrios metabólicos são sugeridos como fatores importantes no início e progressão da doença, entre as causas está o fato que muitas vias de sinalização são reguladas de forma redox-sensível (MANGGE et al., 2014). O conhecimento de alterações influenciadas por fatores nutricionais e mutacionais parece ser a chave para o desenvolvimento de terapias para prevenção de doenças e prolongamento do tempo de vida saudável em humanos.

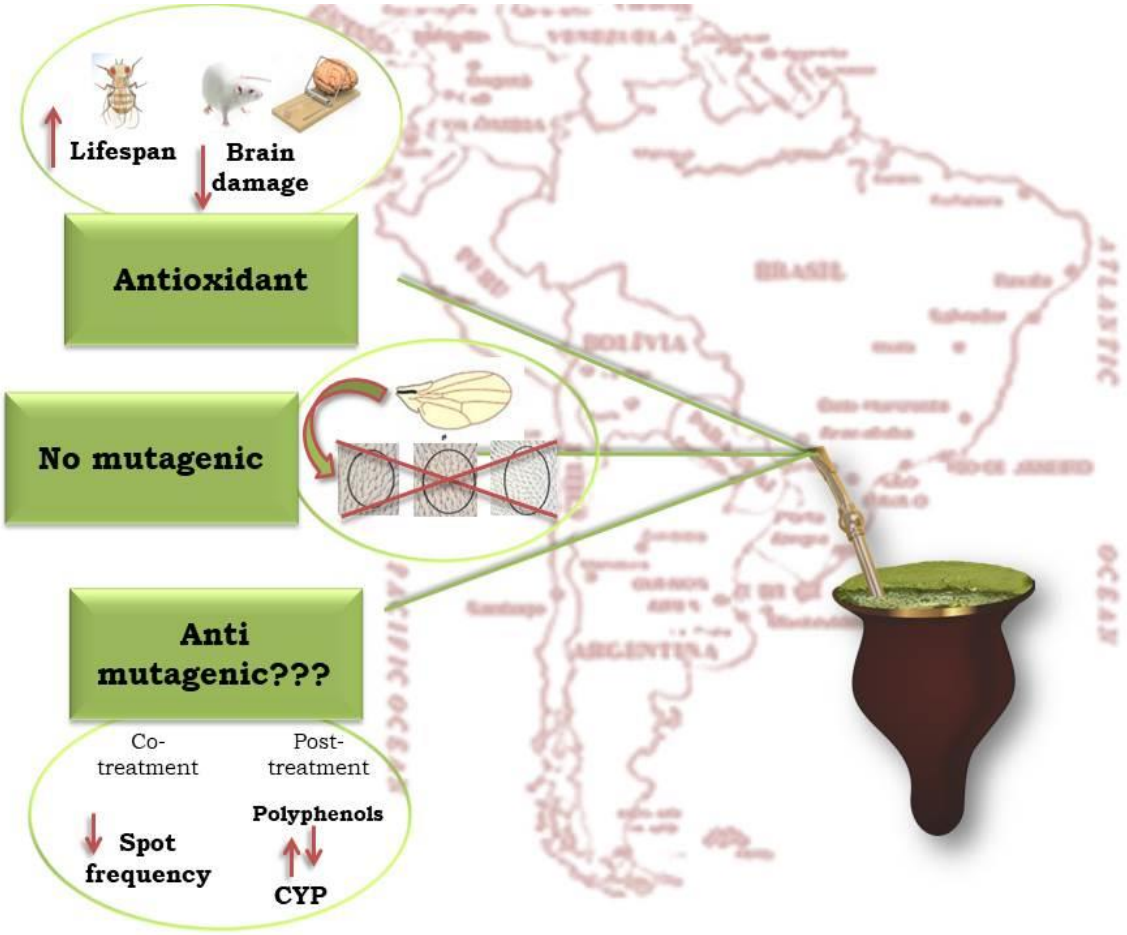


Figura 8- Resumo gráfico dos principais efeitos observados em relação ao uso dos extratos de erva-mate.

6 REFERÊNCIAS

AMIGO-BENAVENT, M.; WANG, S.; MATEOS, R. et al. Antiproliferative and cytotoxic effects of green coffee and yerba mate extracts, their main hydroxycinnamic acids, methylxanthine and metabolites in different human cell lines. **Food and Chemical Toxicology**. vol. 106, p. 125-138, 2017. DOI: 10.1016/j.fct.2017.05.019.

ANDRADE, H. H. R.; REGULY, M. L.; LEHMANN, M. Wing somatic mutation and recombination test. In: **Drosophila Cytogenetics Protocols**. HENDERSON, D. S. (Ed). New Jersey: Humana Press. 2004.

ANDRADE, J. P.; ASSUNÇÃO, M. Protective Effects of Chronic Green Tea Consumption on Age-related Neurodegeneration. **Current Pharmaceutical Design**. vol. 18, n. 1, p. 4-14, 2012.

ARÇARI, D. P.; SANTOS, J. C.; GAMBERO, A. et al. The in vitro and in vivo effects of yerba mate (*Ilex paraguariensis*) extract on adipogenesis. **Food Chemistry**. vol. 141, n. 2, p. 809-15, 2013.DOI: 10.1016/j.foodchem.2013.04.062.

ARUL, D.; SUBRAMANIAN, P. Inhibitory effect of naringenin (*citrus flavonone*) on N-nitrosodiethylamine induced hepatocarcinogenesis in rats. **Biochemical and Biophysical Research Communications**. vol. 434, n. 2, p. 203-209, 2013. DOI: 10.1016/j.bbrc.2013.03.039.

BAINS, Y.; GUGLIUCCI, A. *Ilex paraguariensis* and its main component chlorogenic acid inhibit fructose formation of advanced glycation end products with amino acids at conditions compatible with those in the digestive system. **Fitoterapia**. vol. 117, p. 6-10, March 2017. DOI: 10.1016/j.fitote.2016.12.006.

BARG, M.; REZIN, G. T.; LEFFA, D. D. et al. Evaluation of the protective effect of *Ilex paraguariensis* and *Camellia sinensis* extracts on the prevention of oxidative damage caused by ultraviolet radiation. **Environmental Toxicology and Pharmacology**. vol. 37, n. 1, p. 195-201, 2014. DOI: 10.1016/j.etap.2013.11.028.

BASHEER, L.; KEREM, Z. Interactions between CYP3A4 and Dietary Polyphenols - Review Article. **Oxidative Medicine and Cellular Longevity**. 2015. DOI: 10.1155/2015/854015.

BASTOS, D. H. M.; SALDANHA, L. A.; CATHARINO, R. R. et al. Phenolic antioxidants identified by ESI-MS from Yerba Mate (*Ilex paraguariensis*) and green tea (*Camelia sinensis*) extracts. **Molecules**. vol. 12, n. 3, p. 423-432, 2007.

BHARUCHA, K. N. The epicurean fly: using *Drosophila melanogaster* to study metabolism. **Pediatric Research**. vol. 65, n. 2, p. 132-7, 2009. DOI: 10.1203/PDR.0b013e318191fc68.

BIRBEN, E.; SAHINER, U. M.; SACKESEN, C. et al. Oxidative stress and antioxidant defense. **World Allergy Organization Journal**. vol. 5, n. 1, p. 9–19, 2012. DOI: 10.1097/WOX.0b013e3182439613.

BOTAS, J. *Drosophila* researchers focus on human disease. **Nature Genetics**. vol. 39, n. 5, p. 589–591, 2007. DOI: 10.1038/ng0507-589.

BOYLE, M. P.; KOLBER, B. J.; VOGT, S. K. et al. Forebrain glucocorticoid receptors modulate anxiety-associated locomotor activation and adrenal responsiveness. **Journal of Neuroscience**. vol. 26, n. 7, p. 1971-1978, 2006. DOI: 10.1523/JNEUROSCI.2173-05.2006.

BRACESCO, N.; DELL, M.; ROCHA, A. et al. Antioxidant activity of a botanical extract preparation of *Ilex paraguariensis*, prevention of DNA double- strand breaks in *Saccharomyces Cerevisiae* and human low-density lipoprotein oxidation. **Journal of Alternative and Complementary Medicine**. vol. 9, n. 3, p. 379-387, 2003.

BRACESCO, N.; SANCHEZ, A. G.; CONTRERAS, V. et al. Recent advances on *Ilex paraguariensis* research: Mini Review. **Journal of Ethnopharmacology**. vol. 136, n. 3, p. 378-84, 2011. DOI: 10.1016/j.jep.2010.06.032.

BUJOLD, M.; GOPALAKRISHNAN, A.; NALLY, E. et al. Nuclear receptor DHR96 acts as a sentinel for low cholesterol concentrations in *Drosophila Melanogaster*. **Molecular and Cellular Biology.** vol. 30, n. 3, p. 793-805, 2010. DOI: 10.1128/MCB.01327-09.

BURNS, T.; JONES, H. J.; AMINOFF, M. J. et al. **Sistema Nervoso** – Cérebro. Volume 7 - Parte I. Coleção Netter de Ilustrações Médicas 2ed. São Paulo: Elsevier Brasil, 2014.

CAMPBELL, J. E.; DRUCKER, D. J. Islet α cells and glucagon – critical regulators of energy homeostasis. **Nature Reviews Endocrinology**. vol. 11, p. 329–338, 2015. DOI: 10.1038/nrendo.2015.51.

CARDOZO JR, E. L.; FERRARESE-FILHO, O.; CARDOZO FILHO, L. et al. Methylxanthines and phenolic compounds in mate (*Ilex paraguariensis* St. Hil.) progenies grown in Brazil. **Journal of Food Composition and Analysis**. vol. 20, p. 553–558, 2007.

CHECHI, L. A.; SCHULTZ, G. A produção de erva-mate: um estudo da dinâmica produtiva nos estados do sul do brasil. **Enciclopédia Biosfera**, Centro Científico Conhecer - Goiânia, v. 13, n. 23, p. 16, 2016. DOI: 10.18677/Enciclopedia_Biosfera_2016_002.

CHEN,Y.; TONG Y.; YANG C.; GAN Y.; SUN H.; BI H.; CAO S.; YIN X. Consumption of hot beverages and foods and the risk of esophageal cancer: a meta-analysis of observational studies. **BMC Cancer**. vol. 15: 449, 2015.

CITTADINI, M. C.; CANALIS, A. M.; ALBRECHT, C. et al. Effects of oral phytoextract intake on phenolic concentration and redox homeostasis in murine encephalic regions. **Nutrition Neuroscience**. vol. 18, n. 7, p. 316-22, 2015.DOI: 10.1179/1476830514Y.0000000130.

COELHO, G.C.; GNOATTO S.B.; BASSANI V.L.; SCHENKEL E.P. Quantification of saponins in extractive solution of mate leaves (*Ilex paraguariensis* A. St. Hil.). **J Med Food**. Vol.13, n.2, p. 439-43,V2010. DOI: 10.1089/jmf.2009.0046.

COLÍN-GONZÁLEZ, A. L.; BECERRÍL, H.; FLORES-REYES, B. R. et al. Acute restraint stress reduces hippocampal oxidative damage and behavior in rats: effect of S-allyl cysteine. **Life Sciences**. vol. 35, p. 165-72, 2015. DOI: 10.1016/j.lfs.2015.06.009.

COLPO, A. C. **Perfil fitoquímico e capacidade antioxidante de extratos de erva-mate (*Ilex paraguariensis A.St. Hill*.).** Universidade Federal do Pampa. Uruguaiana/RS, 2012. Disponível em: <http://cursos.unipampa.edu.br/cursos/ppgbioq/files/2012/04/Perfil-fitoqu%C3%ADmico-e-capacidade-antioxidante-de-extratos-de-erva-mate-Ilex-paraguariensis-A.-St.-Hill.2.pdf >. Acesso em: 25 de março de 2017.

COLPO, A. C.; ROSA, H.; LIMA, M. E. et al. Yerba mate (*Ilex paraguariensis* St. Hill.)-based beverages: How successive extraction influences the extract composition and its capacity to chelate iron and scavenge free radicals. **Food Chemistry**. vol. 209, p. 185-95, 2016. DOI: 10.1016/j.foodchem.2016.04.059.

COLPO, G.; TREVISOL, F.; TEIXEIRA, A. M. et al. *Ilex paraguariensis* has antioxidant potential and attenuates haloperidol-induced orofacial dyskinesia and memory dysfunction in rats. **Neurotoxicity Research**. vol. 12, n. 3, p. 171-80, 2007.

DAI, J.; MUMPER, R. J. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. **Molecules**. vol. 15, p. 7313-7352, 2010. DOI: 10.3390/molecules15107313.

DOKE, S. K.; DHAWAL, S. C. Alternatives to animal testing: a review. **Saudi Pharmaceutical Journal**. vol. 23, n. 3, p. 223-229, 2015. DOI: 10.1016/j.jsps.2013.11.002.

DURAYSKI, J. **Tomas um mate?** Uma análise da cultura de consumo do chimarrão em um contexto urbano. Universidade do Vale do Rio dos Sinos. São Leopoldo/RS, 2013.

EDIFIZI, D.; SCHUMACHER, B. Genome Instability in Development and Aging: Insights from Nucleotide Excision Repair in Humans, Mice, and Worms. **Biomolecules**. vol. 5, n. 3, p. 1855–1869, 2015. DOI: 10.3390/biom5031855.

ERVA-MATE combate colesterol ruim, diabetes e até emagrece. In: **Globo Repórter – g1.globo.com**. 2012. **Disponível em:** <http://g1.globo.com/globo-reporter/noticia/2012/02/erva-mate-combate-colesterol-ruim-diabetes-e-ate-emagrece.html>. **Acesso em: 24 de junho de 2017.**

FINKEL, T.; HOLBROOK, N. J. Oxidants, oxidative stress and the biology of ageing. **Nature**. vol. 408, p. 239-247, 2000. DOI: 10.1038/35041687.

FONSECA, C. A.; OTTO, S. S.; PAUMGARTTEN, F. J. et al. Nontoxic, mutagenic, and clastogenic activities of Mate-Chimarrão (*Ilex paraguariensis*). **Journal of Environmental Pathology, Toxicology and Oncology**. vol. 19, n. 4, p. 333-46, 2000.

FONSECA, T. R. **Efeitos do exercício físico e do extrato de soja no perfil lipídico, no estresse oxidativo e na aterosclerose em camundongos deficientes do gene para o receptor de LDL.** Universidade Federal de Minas Gerais. Belo Horizonte/MG, 2007.

FREI, H.; WÜRGLER, F.E.Statistical methods to decide whether mutagenicity test data from *Drosophila* assays indicate a positive, negative or inconclusive result. **Mutat. Res.** vol. 203, p 297-308, 1988.

FRÖLICH, A.; WÜRGLER, F. E. New tester strains with improved bioactivation capacity for the *Drosophila* wing spot test. **Mutation Research**. vol. 216, n. 3, p. 179-187, 1989.

GALLEY, H. F. Oxidative stress and mitochondrial dysfunction in sepsis. **British Journal of Anaesthesia**. vol. 107, n. 1, p. 57-64, 2011. DOI: 10.1093/bja/aer093.

GENECARDS. The Human Gene Database. **NPC1 like intracellular cholesterol transporter 1**. Disponível em: < http://www.genecards.org/Search/Keyword?queryString=NPC1%20like%20intracellular%20cholesterol%20transporter%201),>. Acesso em: 13 de março de 2017.

GILBERT, L. I.; RYBCZYNSKI, R.; WARREN, J. T. Control and biochemical nature of the ecdysteroidogenic pathway. **Annual Review of Entomology**. vol. 47, p. 883–916, 2002. DOI: 10.1146/annurev.ento.47.091201.145302.

GLAVIN, G. B.; PARÉ, W. P.; SANDBAK, T. et al. Restraint stress in biomedical research: an update. **Neuroscience & Biobehavioral Reviews**. vol. 18, n. 2, p. 223-249, 1994.

GNOATTO, S. C. B.; BASSANI, V. L.; COELHO, G. C. et al. Influência do método de extração nos teores de metilxantinas em erva-mate (*Ilex paraguariensis A. ST.-HIL., aquifoliaceae).* **Química Nova**. vol. 30, p. 304-307, 2007.

GOSMANN, G.; GUILLAUME, D.; TAKETA, A. T. et al. Triterpenoid saponins from *Ilex paraguariensis*. **Journal of Natural Products**. vol. 58, n. 3, p. 438-41, 1995.

GOSMANN, G.; SCHENKEL, E. P.; SELIGNMANN, O. A New Saponin from Mate, *Ilex paraguariensis*. **Journal of Natural Products**. vol. 52, n. 6, p. 1367-1370, 1989. DOI: 10.1021/np50066a036.

GRAF, U.; FREI, H.; KÄGI, A. et al. Thirty compounds tested in the *Drosophila* wing spot test. **Mutation Research**. vol. 222, n. 4, p. 359-73, 1989.

GRAF, U.; WÜRGLER, F. E.; KATZ, A. J. et al. Somatic mutation and recombination test in *Drosophila melanogaster*. **Environmental Mutagenesis**. vol. 6, n. 2, p. 153-188, 1984.

GUPTA, R. K.; PATEL, A. K.; SHAH, N. et al. Oxidative stress and antioxidants in disease and cancer: a review. **Asian Pacific Journal of Cancer Prevention**. vol. 15, n. 11, p. 4405-9. 2014. DOI: 10.7314/APJCP.2014.15.11.4405.

GUTTERIDGE, J. M.; HALLIWELL, B. Antioxidants: Molecules, medicines, and myths. **Biochemical and Biophysical Research Communications**. vol. 393, n. 4, p. 561-4, 2010. DOI: 10.1016/j.bbrc.2010.02.071.

HALL, S.; DESBROW, B.; ANOOPKUMAR-DUKIE, S. A review of the bioactivity of coffee, caffeine and key coffee constituents on inflammatory responses linked to depression. **Food Research International.** vol. 76, p. 626-636, 2015. DOI: 10.1016/j.foodres.2015.07.027.

HALLIWELL, B.; GUTTERIDE, J. M. C. **Free radicals in biology and medicine**. 4ed. USA: Oxford University Press. 2007. cap. 3, 4, 5.

HALLIWELL, B. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. **Drugs & Aging**. vol. 18, n. 9, p. 685-716, 2001.

HECK, C. I.; MEJIA, E. G. Yerba mate tea (*Ilex paraguariensis*): a comprehensive review on chemistry, health implications, and technological considerations. **Journal of Food Science**. v. 72, p. 138-151, 2007. DOI: 10.1111/j.1750-3841.2007.00535.x.

HEINZMANN, B. M.; SCHENKEL, E. P. Saponins from *Ilex dumosa*. **Journal of Natural Products**. vol. 58, n. 9, p. 1419-1422, 1995. DOI: 10.1021/np50123a012.

HEITMAN, E.; INGRAM, D. K. Cognitive and neuroprotective effects of chlorogenic acid. **Nutritional Neuroscience**. vol. 20, n. 1, p. 32-39, 2016. DOI: 10.1179/1476830514Y.0000000146.

HOLMSTRÖM, K. M.; FINKEL, T. Cellular mechanisms and physiological consequences of redox-dependent signalling. **Nature Reviews Molecular Cell Biology**. vol. 15, n. 6, p. 411-21, 2014. DOI: 10.1038/nrm3801.

HORNER, M. A.; PARDEE, K.; LIU, S. et al. The *Drosophila* DHR96 nuclear receptor binds cholesterol and regulates cholesterol homeostasis. **Genes and Development**. vol. 23, n. 23, p. 2711–2716, 2009. DOI: 10.1101/gad.1833609.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA (IBGE). Produção da Extração Vegetal e da Silvicultura 2013. Disponível em: <http://www.ibge.gov.br/home/estatistica/economia/pevs/2013/default.shtm>. Acesso em: 24 de junho de 2017.

INSTITUTO NACIONAL DE LA YERBA MATE (INYM). **Convenio histórico entre la carne, el vino y la yerba mate argentina**. April 25, 2017. Disponível em: <http://www.inym.org.ar/en/2017/04/25/convenio-historico-entre-la-carne-el-vino-y-la-yerba-mate-argentina/>. Acesso em: 25 de junho de2017.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC). Coffee, tea, mate, methylxanthines and methylglyoxal. In: **IARC Monographs on the evaluation of carcinogenic risks to humans**. vol. 51. Lyon, France: IARC, 1991. Disponível em: < http://monographs.iarc.fr/ENG/Monographs/vol51/index.php>. Acesso em: 17 de julho de 2017.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC). Review of Human Carcinogens. In: **IARC Monographs on the evaluation of carcinogenic risks to humans**. vol. 100. 2016. Disponível em:

<http://apps.who.int/bookorders/anglais/detart1.jsp?sesslan=1&codlan=1&codcol=72&codcch=100>. Acesso em: 10 de julho de 2017.

ISOLABELLA, S.; COGOI, L.; LÓPEZ P. et al. Study of the bioactive compounds variation during yerba mate (*Ilex paraguariensis*) processing. **Food Chemistry**. vol. 122, p. 695–699, 2010.

JAN, A. T.; AZAM, M.; SIDDIQUI, K. et al. Heavy metals and human health: mechanistic insight into toxicity and counter defense system of antioxidants. **International Journal of Molecular Sciences.** vol. 16, n. 12, p. 29592–29630, 2015. DOI: 10.3390/ijms161226183.

KARDINAAL, A. F.; VAN ERK, M. J.; DUTMAN, A. E. et al. Quantifying phenotypic flexibility as the response to a high-fat challenge test in different states of metabolic health. **The FASEB Journal**. vol. 29, n. 11, p. 4600-13, 2015. DOI: 10.1096/fj.14-269852.

KHAN, N.; MUKHTAR, H. Tea polyphenols for health promotion. **Life Sciences**. vol. 81, n. 7, p. 519-533, 2007. DOI: 10.1016/j.lfs.2007.06.011.

KING-JONES, K.; HORNER, M. A.; LAM, G. et al. The DHR96 nuclear receptor regulates xenobiotic responses in *Drosophila*. **Cell Metabolism**. vol. 4, n. 1, p. 37–48, 2006. DOI: 10.1016/j.cmet.2006.06.006.

KOROBKOVA, E. A. Effect of natural polyphenols on cyp metabolism: implications for diseases. **Chemical Research in Toxicology**. vol. 28, n. 7, p. 1359-90, 2015. DOI: 10.1021/acs.chemrestox.5b00121.

KRAEMER, K. H.; TAKETA, A. T.; SCHENKEL, E. P. et al. Matesaponin 5, a highly polar saponin from *Ilex paraguariensis*. **Phytochemistry**. vol. 42, n. 4, p. 1119-1122, 1996.

KRYCH-MADEJ, J.; GEBICKA, L. Interactions of nitrite with catalase: enzyme activity and reaction kinetics studies. **Journal of Inorganic Biochemistry**. vol. 171, p. 10–17, 2017. DOI: 10.1016/j.jinorgbio.2017.02.023.

KYSELOVA, Z. Toxicological aspects of the use of phenolic compounds in 389 disease prevention. **Interdisciplinary Toxicology**. vol. 4, n. 4, p. 173-183, 2011. DOI: 10.2478/v10102-011-0027-5.

LEÓN-GONZÁLEZ, A. J.; AUGER, C.; SCHINI-KERTH, V. B. Pro-oxidant activity of polyphenols and its implication on cancer chemoprevention and chemotherapy. **Biochemical Pharmacology**. vol. 98, n. 3, p. 371-380, 2015. DOI: 10.1016/j.bcp.2015.07.017.

LEOPOLDINI, M.; RUSSO, N.; TOSCANO, M. The molecular basis of working 563 mechanism of natural polyphenolic antioxidants. **Food Chemistry**. vol. 125, n. 2, p. 288-306, 2011. DOI: 10.1016/j.foodchem.2010.08.012.

LIANG, N.; KITTS, D. D. Role of Chlorogenic Acids in Controlling Oxidative and Inflammatory Stress Conditions. **Nutrients**. vol. 8, p. 16, 2015. DOI: 10.3390/nu8010016.

LIMA, M. E.; COLPO, A. C.; SALGUEIRO, W. G. et al. *Ilex paraguariensis* extract increases lifespan and protects against the toxic effects caused by paraquat in *Caenorhabditis elegans*. **International Journal of Environmental Research and Public Health**. vol. 1, n. 10, p. 10091-10104, 2014. DOI: 476 10.3390/ijerph111010091.

LIN, L. C.; WANG, M. N.; TSENG, T. Y. et al. Pharmacokinetics of (-)-epigallocatechin-3-gallate in conscious and freely moving rats and its brain regional distribution. **Journal of Agricultural and Food Chemistry**. vol. 55, n. 4, p. 1517-24, 2007.

LINDVALL, O.; AUER, R. N.; SIESJÖ, B. K. Selective lesions of mesostriatal dopamine neurons ameliorate hypoglycemic damage in the caudate-putamen. **Experimental Brain Research**. vol. 63, n. 2, p. 382-386, 1986.

LISTON, C.; MILLER, M. M.; GOLDWATER, D. S. et al. Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. **Journal of Neuroscience**. vol. 26, p. 7870-7874, 2006. DOI: 10.1523/JNEUROSCI.1184-06.2006.

LIU, J.; WANG, X.; SHIGENAGA, M. K. et al. Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. **The FASEB Journal.** vol. 10, n. 13, p. 1532-8, 1996.

LIU, N.; WANG, L. H.; GUO, L. L. et al. Chronic restraint stress inhibits hair growth via substance P mediated by reactive oxygen species in mice. **Plos One**. vol. 8, n. 4, 2013. DOI: 10.1371/journal.pone.0061574.

LUBIN, J. H.; STEFANI, E. de; ABNET, C. C. et al. Maté drinking and esophageal squamous cell carcinoma in south america: pooled results from two large multicenter case–control studies. **Cancer Epidemiology, Biomarkers & Prevention**. vol. 23, n. 1, p. 107-16, 2014. DOI: 10.1158/1055-9965.EPI-13-0796.

LUCASSEN, P. J.; PRUESSNER, J.; SOUSA, N. et al.Neuropathology of stress. **Acta Neuropathologica**. vol. 127, n. 1, p. 109-135, 2014. DOI: 10.1007/s00401-013-1223-5.

MANGGE, H.; BECKER, K.; FUCHS, D. et al. Antioxidants, inflammation and cardiovascular disease. **World Journal of Cardiology**. vol. 6, n. 6, p. 462-477, 2014. DOI: 10.4330/wjc.v6.i6.462.

MCEWEN, B. S.; GRAY, J. D.; NASCA, C. Recognizing resilience: Learning from the effects of stress on the brain. **Neurobiology of Stress**. vol. 1, p. 1-11, 2015. DOI: 10.1016/j.ynstr.2014.09.001.

MEINHART, A. D.; BIZZOTTO, C. S.; BALLUS, C. A. et al. Methylxanthines and phenolics content extracted during the consumption of mate (*Ilex paraguariensis* St. Hil) beverages. **Journal of Agricultural and Food Chemistry**. vol. 58, n. 4, p. 2188-93, 2010. DOI: 10.1021/jf903781w.

MÉNDEZ-CUESTA, L. A.; MÁRQUEZ-VALADEZ, B.; PÉREZ-DE LA CRUZ, V. et al. Early changes in oxidative stress markers in a rat model of acute stress: effect of L-carnitine on the striatum. **Basic & Clinical Pharmacology & Toxicology**. vol. 109, n. 2, p. 123-129, 2011. DOI: 10.1111/j.1742-7843.2011.00691.x.

MIRANDA, D. D.; ARÇARI, D. P.; PEDRAZZOLI Jr, J. et al. Protective effects of mate tea (*Ilex paraguariensis*) on H2O2-induced DNA damage and DNA repair in mice. **Mutagenesis**. vol. 23, n. 4, p. 261-5, 2008. DOI: 10.1093/mutage/gen011.

MISRA, J. R.; HORNER, M. A.; LAM, G. et al. Transcriptional regulation of xenobiotic detoxification in *Drosophila*. **Genes & Development**. vol. 25, n. 17, p. 1796-806, 2011. DOI: 10.1101/gad.17280911.

MUÑOZ-CULLA, M.; SÁENZ-CUESTA, M.; GUERECA-BARANDIARAN, M. J. et al. Yerba mate (*Ilex paraguariensis*) inhibits lymphocyte activation in vitro. **Food Funct**. vol. 7, p. 11, p. 4556-4563, 2016.

NIWA, R.; NIWA, Y. S. The fruit fly *Drosophila melanogaster* as a model system to study Cholesterol metabolism and homeostasis. **Cholesterol**. vol. 2011, p. 1-6, 2011. DOI: 10.1155/2011/176802.

OISHI, Y.; MANABE, I. Integrated regulation of the cellular metabolism and function of immune cells in adipose tissue. **Clinical and Experimental Pharmacology and Physiology**. vol. 43, n. 3, p. 294–303. 2016. DOI: 10.1111/1440-1681.12539.

OTANI, H. Site-specific antioxidative therapy for prevention of atherosclerosis and cardiovascular disease. **Oxidative Medicine and Cellular Longevity**. vol. 2013, p. 1-14, 2013. DOI: 10.1155/2013/796891.

PARAGUAY. Ministerio de agricultura y Ganadería. **Unidad de Estudios Agroeconomicos. Zonificacion Agroecologica de Rubros Agropecuarios del Paraguay**. Disponível em: <http://www.mag.gov.py/dgp/ZONIFICACION%20AGROECOLOGICA%20ZAFRA%202012%202013.pdf>. Acesso em: 24 de junho de 2017.

PENG, C.; CHAN, H. Y.; LI, Y. M. et al. Black tea theaflavins extend the lifespan of fruit flies. **Experimental Gerontology**. vol. 44, n. 12, p. 773-783, 2009. DOI: 10.1016/j.exger.2009.09.004.

PEREIRA, A. A. F.; TIRAPELIA, K. G.; CHAVES-NETO, A. H. et al. *Ilex paraguariensis* supplementation may be an effective nutritional approach to modulate oxidative stress during perimenopause. **Experimental Gerontology.** vol. 90, p. 14–18, 2017. DOI: 10.1016/j.exger.2017.01.011.

PEREIRA, D. F.; KAPPELA, V. D.; CAZAROLLI, L. H. et al. Influence of the traditional Brazilian drink *Ilex paraguariensis* tea on glucose homeostasis. **Phytomedicine**. vol. 19, p. 868– 877, 2012.

PESQUISA inédita na Urcamp relaciona a erva-mate à longevidade. **Jornal Minuano**, Bagé, 06 jan. 2016. Ano XXI, n. 5.233.

POPA-WAGNER, A.; MITRAN, S.; SIVANESAN, S. et al. ROS and brain diseases: the good, the bad, and the ugly - Review Article. **Oxidative Medicine and Cellular Longevity**. 2013. DOI: 10.1155/2013/963520.

PRAKASH, G.; HOSETTI, B.; DHANANJAYA, B. Antimutagenic effect of Dioscorea Pentaphylla on genotoxic effect induced by methyl methanesulfonate in the *drosophila* wing spot test. **Toxicology International**. vol. 21, n. 3, p. 258, 2014. DOI: 10.4103/0971-6580.155341.

PUANGPRAPHANT, S.; BERHOW, M. A.; MEJIA, E. G. Mate (*Ilex paraguariensis St. Hilaire*) saponins induce caspase-3-dependent apoptosis in human colon cancer cells in vitro. **Food Chemistry**. vol. 125, p. 1171-1178, 2011.

PUANGPRAPHANT, S.; DIA, V. P.; MEJIA, E. G. de, et al. Yerba mate tea and mate saponins prevented azoxymethane-induced inflammation of rat colon through suppression of NF-κB p65ser(311) signaling via IκB-α and GSK-3β reduced phosphorylation. **Biofactors**. vol. 39, n. 4, p. 430-40, 2013. DOI: 10.1002/biof.1083.

RAND, M. D.; LOWE, J. A.; MAHAPATRA, C. T. *Drosophila* CYP6g1 and its human homolog CYP3A4 confer tolerance to methylmercury during development. **Toxicology**. vol. 300, n. 1-2, p. 75-82, 2012. DOI: 10.1016/j.tox.2012.06.001.

RANGANATHA, N.; KUPPAST, I. J. A review on alternatives to animal testing methods in drug development. **International Journal of Pharmacy and Pharmaceutical Sciences**. vol. 4, n. 5, p. 28-32, 2012.

RAZONES de un hábito nacional: especialistas explican que la yerba mate no es adictiva, pero sí un estimulante que genera dependencia física además de ser un hábito cultural. **El Observador**, septiembre 20, 2013. Disponível em: < http://www.elobservador.com.uy/razones-un-habito-nacional-n260661>. Acesso em: 24 de junho de 2017.

REGE, S. D.; GEETHA, T.; GRIFFIN, G. D. et al. Neuroprotective effects of resveratrol in Alzheimer disease pathology. **Frontiers in Aging Neuroscience**. vol. 6, p. 218. 2014. DOI: 10.3389/fnagi.2014.00218.

RESENDE, P. E. de; VERZA, S. G.; KAISER, S. et al. The activity of mate saponins (*Ilex paraguariensis*) in intra-abdominal and epididymal fat, and glucose oxidation in male Wistar rats. **Journal of Ethnopharmacology**. vol. 144, n. 3, p. 735-740, 2012. DOI: 10.1016/j.jep.2012.10.023.

RIBEIRO, L. R.; SALVADORI, D. M. F.; MARQUES, E. K. **Mutagênese ambiental**. Canoas: Editora da Ulbra, 2003.

RODRIGUES, C. R.; PLENTZ, L. C.; FLORES, M. D. Assessment of genotoxic and antigenotoxic activities of artepillin C in somatic cells of Drosophila melanogaster. **Food Chemical Toxicology**. vol. 101, p. 48-54, 2017. DOI: 10.1016/j.fct.2017.01.005.

RODRIGUES, C. R.; PLENTZ, L. C.; MARCUCCI, M. C. et al. In vivo evaluation of mutagenic and recombinagenic activities of Brazilian propolis. **Food Chemical Toxicology.** vol. 96, p. 117-21, 2016. DOI: 10.1016/j.fct.2016.07.037.

SAHIN, E.; GUMUSLU, S. Immobilization stress in rat tissues: alterations in protein oxidation, lipid peroxidation and antioxidant defenses system. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology.** vol. 144, n. 4, p. 342-347, 2007.

SAINT-HILAIRE, A. **Viagem pela comarca de Curitiba**. Tradução de Cassiana Lacerda Carollo. Curitiba: Fundação Cultural de Curitiba, 1995.

SAMARGHANDIAN, S.; AZIMI-NEZHAD, M.; BORJI, A. et al. Protective effects of carnosol against oxidative stress induced brain damage by chronic stress in rats. **BMC Complementary and Alternative Medicine**. vol. 17, n. 1, p. 249, 2017. DOI: 10.1186/s12906-017-1753-9.

SANER, C.; WEIBEL, B.; WÜRGLER, F. E. et al. Metabolism of promutagens catalyzed by *Drosophila melanogaster* CYP6A2 enzyme in *Saccharomyces cerevisiae*. **Environmental and Molecular Mutagenesis**. vol. 27, n. 1, p. 46-58, 1996.

SANTOS, L. dos. **A planta que fez um estado prosperar**. Jornal Gazeta do povo [s/d]. Disponível em: <http://www.gazetadopovo.com.br/vida-e-cidadania/especiais/erva-mate/index.jpp>. Acesso em: 24 de junho de 2017.

SANTOS, T. B.; MITCHELL, D. A.; KRIEGER, N. et al. Experimental and modeling study of enzymatic oxidation of 5-o-caffeoylquinic acid by polyphenol oxidases. **Food Technology and Biotechnology.** vol. 48, p. 548-553, 2010.

SAPOLSKY, R. M. Glucocorticoid toxicity in the hippocampus: reversal by supplementation with brain fuels. **Journal of Neuroscience**. vol. 6, n. 8, p. 2240-4, 1986.

SCHAFER, F. Q.; BUETTNER, G. R. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. **Free Radical Biology & Medicine**. vol. 30, n. 11, p. 1191-212, 2001.

SCHAFFER, S.; HALLIWELL, B. Do polyphenols enter the brain and does it matter? Some theoretical and practical considerations. **Genes Nutrition**. vol. 7, n. 2, p. 99-109, 2012. DOI: 10.1007/s12263-011-0255-5.

SCHEEPENS, A.; TAN K.; PAXTON J.W. Improving the oral bioavailability of beneficial polyphenols through designed synergies. **Genes Nutrition** . vol. 5, n.1, p 75-87, 2010. DOI: 10.1007/s12263-009-0148-z.

SCHINELLA, G.; NEYRET, E.; CÓNSOLE, G. et al. An aqueous extract of *Ilex paraguariensis* reduces carrageenan-induced edema and inhibits the expression of cyclooxygenase-2 and inducible nitric oxide synthase in animal models of inflammation. **Planta Medica**. vol. 80, n. 12, p. 961-8, 2014. DOI: 10.1055/s-0034-1382876.

SHEEHAN, D.; MEADE, G.; FOLEY, V. M. et al. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. **Biochemical Journal.** vol. 15, p. 1–16, 2001.

SIEBER, M. H.; THUMMEL, C. S. The DHR96 nuclear receptor controls triacylglycerol homeostasis in *Drosophila*. **Cell Metabolism**. vol. 10, p. 481–490, 2009. DOI: 10.1016/j.cmet.2009.10.010.

SILVA, R. A. da; DIHL, R. R.; DIAS, L. P. et al. DNA damage protective effect of honey-sweetened cashew apple nectar in *Drosophila melanogaster*. **Genetics and Molecular Biology**. vol. 39, n. 3, p. 431–441, 2016. DOI: 10.1590/1678-4685-GMB-2015-0129.

SKORUPA, D. A.; DERVISEFENDIC, A.; ZWIENER, J. et al. Dietary composition specifies consumption, obesity, and lifespan in *Drosophila melanogaster*. **Aging Cell**. vol. 7, n. 4, p. 478-90, 2008. DOI: 10.1111/j.1474-9726.2008.00400.x.

SOTO-VACA, A.; GUTIERREZ, A.; LOSSO, J. N. et al. Evolution of phenolic compounds from color and flavor problems to health benefits. **Journal of Agricultural and Food Chemistry**. v. 60, p. 6658−6677, 2012. DOI: 10.1021/jf300861c.

SPENCER, J. P.; VAFEIADOU, K.; WILLIAMS, R. J. et al. Neuroinflammation: modulation by flavonoids and mechanisms of action. **Molecular Aspects of Medicine**. vol. 33, n. 1, p. 83-97, 2012. DOI: 10.1016/j.mam.2011.10.016.

SUGIMOTO, S.; NAKAMURA, S.; YAMAMOTO, S. et al. Brazilian natural medicines. III. structures of triterpene oligoglycosides and lipase inhibitors from mate leaves of *Ilex paraguariensis*. **Chemical & Pharmaceutical Bulletin**. vol. 57, n. 3, p. 257–261, 2009.

TANG, X.; LUO, Y. X.; CHEN, H. Z. et al. Mitochondria, endothelial cell function, and vascular diseases. **Frontiers in Physiology**. vol. 5, p. 175, 2014. DOI: 10.3389/fphys.2014.00175.

TANTAWY, A. A.; ADLY, A. A.; ISMAIL, E. A. et al. Endothelial nitric oxide synthase gene intron 4 VNTR polymorphism in sickle cell disease: relation to vasculopathy and disease severity. **Pediatric Blood & Cancer**. vol. 62, n. 3, p. 389-94, 2015. DOI: 10.1002/pbc.25234.

TATAR, M.; POST, S.; YU, K. Nutrient control of *Drosophila longevity*. **Trends in Endocrinology & Metabolism**. vol. 25, n. 10, p. 509-517, 2014. DOI: 10.1016/j.tem.2014.02.006.

TENNESSEN J.M.; BARRY W.E.; COX J.; THUMMEL C.S. Methods for studying metabolism *in Drosophila*. **Methods.** Vol. 68, n.1, p.105-115, 2014. DOI: 10.1016/j.ymeth.2014.02.034.

THEA, A. E.; FERREIRA, D.; BRUMOVSKY, L. A. et al. Polycyclic aromatic hydrocarbons (PAHs) in yerba maté (*Ilex paraguariensis* St. Hil) traditional infusions (*mate* and *tereré*). **Food Control**. vol. 60, p. 215-220, 2016. DOI: 10.1016/j.foodcont.2015.07.046.

TRINH, I.; BOULIANNE, G. L. Modeling obesity and its associated disorders in *Drosophila*. **Physiology (Bethesda)**. vol. 28, n. 2, p. 117-124, 2013. DOI: 10.1152/physiol.00025.2012.

VAUZOUR, D. Dietary polyphenols as modulators of brain functions: biological actions and molecular mechanisms underpinning their beneficial effects. **Oxidative Medicine and Cellular Longevity**. 2012. DOI: 10.1155/2012/914273.

VAUZOUR, D . Polyphenols and brain health. **OCL Oilseeds & fats Crops and Lipids**. vol. 24, n. 2, 2017. DOI: 10.1051/ocl/2017008.

VIJG, J.; SUH, Y. Genome instability and aging. **Annual Review of Physiology**. vol. 75, p. 645-668, 2013. DOI: 10.1146/annurev-physiol-030212-183715.

VYAS, A.; MITRA, R.; SHANKARANARAYANA RAO, B. S. et al. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. **Journal of Neuroscience**. vol. 22, n. 15, p. 6810-6818, 2002. DOI: 20026655.

WANG, L.; LI, Y. M.; LEI, L. et al. Cranberry anthocyanin extract prolongs lifespan of fruit flies. **Experimental Gerontology**. vol. 69, p. 189-195, 2015. DOI: 10.1016/j.exger.2015.06.021.

WANG, X.; MICHAELIS, E. K. Selective neuronal vulnerability to oxidative stress in the brain. **Frontiers in Aging Neuroscience**. vol. 2, p. 12, 2010. DOI: 10.3389/fnagi.2010.00012.

WNUK, M.; LEWINSKA, A.; OKLEJEWICZ, B. et al. Evaluation of the cyto- and genotoxic activity of yerba mate (*Ilex paraguariensis*) in human lymphocytes in vitro. **Mutation Research**. vol. 679, n. 1-2, p. 18-23, 2009. DOI: 10.1016/j.mrgentox.2009.07.017.

YANG, R. L.; SHI, Y. H.; HAO, G. et al. Increasing Oxidative Stress with Progressive Hyperlipidemia in Human: Relation between Malondialdehyde and Atherogenic Index. **Journal of *Clinical Biochemistry* and *Nutrition***. vol. 43, n. 3, p. 154-158, 2008. DOI: 10.3164/jcbn.2008044.

YOUDIM, K. A.; DOBBIE, M. S.; KUHNLE, G. et al. Interaction between flavonoids and the blood-brain barrier: in vitro studies. **Journal of Neurochemistry**.; vol. 85, n. 1, p. 180-92, 2003.

ZAIDI, S. M. K. R.; AL-QIRIM, T. M.; BANU, N. Effect of antioxidant vitamins on glutathionedepletion and lipid peroxidation induced by restraint stress in the rat liver. **Drug in R&D**. vol. 6, n. 3, p. 157-165, 2006.