UNIVERSIDADE FEDERAL DO PAMPA

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AÇÃO DE *Senecio brasiliensis* (SPRENG) LESS SOBRE A TAXA DE ECLOSÃO E MODULAÇÃO DE MARCADORES DE TOXICIDADE EM LARVAS DE *Drosophila melanogaster*

São Gabriel
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Dissertação apresentada ao Programa de Pós-graduação *Stricto sensu* em Ciências Biológicas da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Mestre em Ciências Biológicas.

Orientador: Thaís Posser

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Ao meu amado pai Zilmar Pereira Macedo (*in memorian*) por ser meu maior exemplo de força, alegria, bondade, coragem e honestidade.

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“Cada avanço da ciência depende de uma nova ousadia da imaginação”.

Walter Waeny

RESUMO

O Brasil apresenta uma rica biodiversidade, sendo que muitas das espécies aqui encontradas tem servido como fonte de compostos com propriedades únicas tanto na medicina como na área biotecnológica. Muitos destes compostos são sintetizados a partir do metabolismo secundário vegetal e nas plantas atuam como atrativos ou mecanismos de defesa. Pesquisas relacionadas com os potenciais das plantas são necessárias para um melhor entendimento das propriedades biológicas que apresentam. *Senecio brasiliensis* (Spreng) Less., é uma espécie vegetal conhecida popularmente como "maria mole", presente em pastagens da região Sul do país, sendo suas folhas consumidas pelos animais e capaz de causar hepatotoxicidade. Tal toxicidade se deve ao seu metabolismo secundário, responsável pela síntese de metabólitos tóxicos, principalmente os alcaloides pirrolizidínicos. A espécie é utilizada popularmente para fins medicinais, e possui demonstrada ação tóxica em insetos. Em vista de sua toxicidade e potencial biotecnológico, um estudo mais completo de sua ação se faz necessário. Neste trabalho avaliou-se o efeito biológico da exposição do extrato hidroalcoólico das folhas de *Senecio brasiliensis* (EHSB) no modelo experimental de *Drosophila melanogaster*. A análise do perfil fitoquímico do EHSB demonstrou a presença de ácidos fenólicos e flavonoides, e atividade antioxidante *in vitro* através dos ensaios de ABTS, DPPH, fenóis totais e FRAP. Em ensaios *in vivo*, EHSB não causou mortalidade de moscas na fase adulta, entretanto a taxa de eclosão foi significativamente diminuída quando as moscas desenvolveram-se em meio de cultura contendo 1 mg/mL de EHSB. Nas larvas do terceiro ínstar observaram-se diminuição da viabilidade celular, aumento da atividade das enzimas antioxidantes GST e SOD, EROs, e diminuição da CAT e aumento da razão GSH/GSSG. Houve aumento na expressão dos genes Nrf2, TrxR, CAT, Drice e Dilp6, e diminuição da fosforilação de proteínas quinases JNK1/2, ERK2 e $p38^{MAPK}$ e AKT, além de um aumento na clivagem de PARP, em paralelo com aumento da atividade de caspase 3/7. Também observou-se uma diminuição nos níveis de glicose, glicogênio e triglicerídeos. O aumento da expressão gênica de Nrf2, da atividade das enzimas GST e SOD e razão GSH/GSSG servem como um indicativo de um estado de estresse oxidativo ocasionado pelo EHSB e atuação da defesa antioxidante das larvas. A diminuição nos níveis de glicose, glicogênio e triglicerídeos pode indicar uma diminuição na reserva energética necessária para fases posteriores ao desenvolvimento larval, como para a eclosão em indivíduo alado, podendo isto ser ocasionado pela inibição da fosforilação de proteínas de transdução de sinal envolvidas neste processo pela diminuição do ATP. Dessa forma, nossos resultados demonstram a toxicidade do HESB e sua capacidade em induzir

marcadores de estresse oxidativo e apoptose celular, prejudicando o desenvolvimento de larvas de *D. melanogaster* e processo de eclosão de moscas adultas.

Palavras-Chave: Desenvolvimento; maria-mole; MAPK; apoptose; estresse oxidativo; defesa antioxidante.

ABSTRACT

Brazil has a high biodiversity, and many species found here are source of compounds with unique medicinal and biotechnological properties. These compounds are synthetized by plants secondary metabolism and can act as attractive or defensive to other species. Thus, studies with plant species are necessary to knowledge about their biologial properties. *Senecio brasiliensis* (Spreng) Less., is a native species popularly known as "maria mole" and is found in pastures of the south region of the country, its leaves serving as food to animals and are able to cause hepatotoxicity due to its secondary metabolism, responsible for the synthesis of toxic metabolites, such as pirrolizidinic alkaloids. In spite of its documented toxicity, this plant is used by Brazilian folk with medicinal purposes. In this study, we have evaluated the biological effects to hydroalcoholic extract of leaves of *S. brasiliensis* (HESB) exposure in *Drosophila melanogaster* experimental model. The fruit fly *D. melanogaster* is an advantageous alternative model useful for the screening of natural substances. Phytochemical constitution of HESB showed the presence of phenolic acids and flavonoids but when comparing with other species, it presented a lower *in vitro* antioxidant activity by ABTS, DPPH, FRAP assays. Survival and locomotor activity of adult flies was unaltered by extract. Nevertheless, we have observed a significant decreasing in eclosion rate of flies, since its embryonic period at 1 mg/mL concentration of HESB. Biochemical and molecular parameters revealed significant changes in third instar larvae of *D. melanogaster* exposed to 1 mg/mL of HESB such as decreased cell viability, stimulation of the activity of antioxidant enzymes SOD and GST, decreasing of CAT, and increasing in GSH/GSSG ratio. The mRNA expression of Nrf2, TrxR, CAT, Drice and Dilp6 were also significantly up-regulated. Decreasing in the phosphorylation of JNK1/2, ERK2, $p38^{MAPK}$ and AKT protein kinases were verified. Apoptotic cell death was inducted by extract. This fact was attested PARP cleavage, in parallel with increasing of caspases 3/7 activity. The increased expression of Nrf2, augmented activity of GST and SOD enzymes activities, and GSH/GSSG ratio in parallel with induction of ROS formation, is an indicative of a state of oxidative stress caused by the HESB and the action of the antioxidant defense of the larvae. It was also observed a decreasing in glucose, glycogen and triglycerides levels indicating a diminution in the energetic reserve required for later stages of larval development, such as for eclosion of winged individuals. Therefore, our results demonstrated of the HESB toxicity and its capacity to induce of cell stress markers and of apoptotic cell death impairing thus the development of *D. melanogaster* larvae and eclosion process of adult flies.

Keywords: Development; maria-mole; MAPK; apoptosis; oxidative stress; antioxidant defense.

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APRESENTAÇÃO

No item **INTRODUÇÃO**, consta uma breve revisão da literatura sobre os temas trabalhados nesta dissertação. A metodologia realizada e os resultados obtidos que fazem parte desta dissertação estão apresentados sob a forma de manuscrito, que se encontra no item **MANUSCRITO**. No mesmo constam as seções: Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas. O item **CONCLUSÕES**, encontrado no final desta dissertação, apresenta interpretações e comentários gerais sobre os resultados do manuscrito presentes neste trabalho. As **REFERÊNCIAS** referem-se somente às citações que aparecem nos itens **INTRODUÇÃO** e **CONCLUSÕES** desta dissertação.

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1. Introdução

As espécies vegetais constituem importante fonte para pesquisas tanto farmacológicas, fitoterápicas e biotecnológicas. Levando em consideração sua diversidade e riqueza fitoquímica pode-se dizer que o potencial destas espécies são pouco explorados. O uso indiscriminado de plantas para fins terapêuticos sem conhecimento científico, e o interesse por bioprodutos que visem um maior cuidado com o meio ambiente e diminuição de resistência de pragas, colaboram para a extrema necessidade de estudos focados nas propriedades das plantas nativas.

Nesse contexto, *Senecio brasiliensis* (Spreng) Less., uma planta nativa muito presente na região Sul do Brasil, que apesar de ser utilizada popularmente para fins medicinais, é conhecida por sua toxicidade e por causar grandes perdas econômicas. Aliando-se ao fato da redução de sacrifícios animais, principalmente de mamíferos em pesquisa, e crescente uso de modelos alternativos, a mosca da fruta *Drosophila melanogaster* destaca-se como uma importante ferramenta para ensaios biológicos, investigação e compreensão de doenças, de toxicidade e modo de ação de compostos variados.

Assim, investigações a respeito dos mecanismos de toxicidade que *S. brasiliensis* apresenta, são de grande valia para contribuições acerca do seu efeito biológico e demais potenciais.

1.1 Revisão da literatura

1.1.1 Biodiversidade da Flora Brasileira

O Brasil é considerado o país com uma megadiversidade agrupando cerca de 20% do total das espécies encontradas no planeta (BIODIVERSIDADE BRASILEIRA, 2002). Com um extenso território e seis biomas com os mais variados climas, vegetações e relevos, o Brasil é o detentor da flora mais rica do mundo, com aproximadamente 15 a 25% do total de espécies, sendo grande parte destas, endêmicas (JOLY et al., 2011).

O metabolismo das espécies vegetais é o que lhes confere esta grande diversidade, compreendendo a síntese de aminoácidos, proteínas e nucleotídeos, essenciais para a

homeostase do organismo, e de substâncias que possuem enorme variação estrutural e que de fato caracterizam a diversidade vegetal. Tais moléculas se distribuem de maneira restrita entre as espécies, e podem ser classificadas em grupos, sendo estes os flavonoides, terpenoides, e alcaloides, que conferem diferentes propriedades as plantas, como de defesa e sobrevivência, além de servirem como atrativos para outras espécies (CROZIER; CLIFFORD; ASHIHARA, 2006; TAIZ; ZEIGER 2006; VICKERY; VICKERY, 1981).

A flora nacional é uma gigantesca e abundante fonte para bioprospecção de novos compostos, mas mesmo apesar de tamanho potencial e diversidade biológica, pesquisas relacionadas com as propriedades das plantas nativas são escassas, havendo assim, carência de estudos focados nesta área no Brasil (BOLZANI, 2016). Unindo-se a isto, a diversidade de atividades biológicas e propriedades físico-químicas que as plantas apresentam, lhes conferem uma série de potenciais, como medicinais, farmacêuticos, fitoterápicos, cosméticos, alimentícios, antimicrobianos quanto de aplicações de interesse ambiental como repelentes, inseticidas e larvicidas, fazendo delas uma importante fonte de estudos para produtos naturais biologicamente ativos (LU; TANG; LI, 2016; MOJZER et al., 2016; PINTO et al., 2002).

Além disso, a ampla aceitação popular e uso indiscriminado das plantas para fins medicinais, unidos à falta de embasamento científico a respeito das suas propriedades farmacológicas ou toxicológicas, trazem sérios riscos para a saúde da população. A toxicidade que algumas espécies podem apresentar é capaz de gerar reações desconhecidas como alergias, intoxicações (aguda ou crônica), ou até mesmo antagonismo de tratamento (CAMPOS et al., 2016; SILVA; HAHN, 2011). Em conjunto, estes fatos corroboram com a necessidade de uma melhor compreensão acerca dos potenciais dos metabólitos vegetais e da interação destes com o ser humano e demais organismos vivos.

1.1.1.1 Interesse em bioprospecção de novos compostos de origem vegetal

As plantas são constituintes fundamentais da trajetória evolutiva do homem, utilizadas desde o princípio da história da humanidade, tanto para fins terapêuticos, quanto como defensivos naturais contra diversas pragas, fato este que culminou para que as plantas coevoluíssem junto dos ataques patogênicos sofridos, por insetos e/ou microrganismos (CORRÊA; SALGADO, 2011; FIRMO et al., 2011; KRINSKI et al., 2014; MARANGONI; MOURA; GARCIA, 2012; PINTO et al., 2002; THACKER, 2002).

As propriedades apresentadas pelos metabólitos vegetais podem ser alternativas viáveis para o desenvolvimento de compostos como pesticidas naturais, eficazes e menos agressivos ao meio ambiente, podendo ser utilizados na forma de óleos essenciais, pós ou extratos vegetais, ou associados a outras práticas já existentes de controle de pragas, visando à redução dos malefícios causados por produtos químicos ao ambiente (BETTIOL; MORANDI, 2009; MACIEL et al., 2010).

Neste contexto, a busca por novos compostos baseados em plantas nativas se faz de grande importância. Bioprodutos com potenciais larvicidas e inseticidas vem ganhando cada vez mais relevância, tendo em vista o crescente aumento de epidemias causadas por vetores biológicos no Brasil (KRINSKI et al., 2014). A utilização de produtos químicos como método convencional para o controle de vetores pode se tornar ineficaz se aplicado de maneira inadequada e/ou indiscriminada, além de que com o tempo os insetos se tornam capazes de adquirir resistência a estes produtos, podendo tolerar concentrações antes letais. Outro fato negativo é o alto custo necessário para a síntese de novas e diferentes moléculas (MACIEL et al., 2010). Contrapondo-se a isto, metodologias alternativas para o controle de insetos vetores, como repelentes de origem botânica, apresentam inúmeras vantagens, sendo capazes de inibir ou prejudicar a ovoposição, reprodução e alimentação do inseto, além de poderem acarretar uma série de mudanças em seu metabolismo, morfologia e comportamento, alterando seu desenvolvimento normal e levando-o a mortalidade na fase imatura ou adulta (CORRÊA; SALGADO, 2011; GALLO et al., 2002; ROEL, 2001). Além disso, são acessíveis, de fácil obtenção, biodegradáveis possuindo baixa persistência ao ambiente, baixo custo para produção e os vetores apresentam baixa resistência a eles (FURTADO et al, 2005).

1.1.2 Família Asteraceae e o gênero *Senecio*

A família Asteraceae (Compositae) encontra-se amplamente distribuída pelo mundo com aproximadamente 1.700 gêneros e 25.000 espécies. O gênero *Senecio* possui em torno de 2.000 espécies sendo 60 destas encontradas principalmente nas regiões Sul e Sudeste do Brasil (FUNK et al., 2009; MATZENBACHER, 2009; OLIVEIRA et al., 2015; TELES, 2015). Este gênero se destaca devido às propriedades biológicas já descritas para as suas espécies: atividades antiulcerogênica e anti-inflamatória de *Senecio brasiliensis* (SOUZA et al., 2015; TOMA et al., 2004); anti-inflamatória de *Senecio salignus* e *Senecio flammeus* (GONZÁLEZ et al., 2013; XIAO et al., 2014); antibacteriana de *Senecio tenuifolius* (MANUBOLU et al., 2013); antiplasmodial de *Senecio smithioides* (MOLLINEDO et al.,

2015); antioxidante e citotóxica de *Senecio graciliflorus* (LONE et al., 2014); citotóxica de *Senecio delphinifolius* (TIDJANI et al., 2013); antioxidante, hemolítica e citotóxica de *Senecio nutants* (LIZARRAGA et al., 2012).

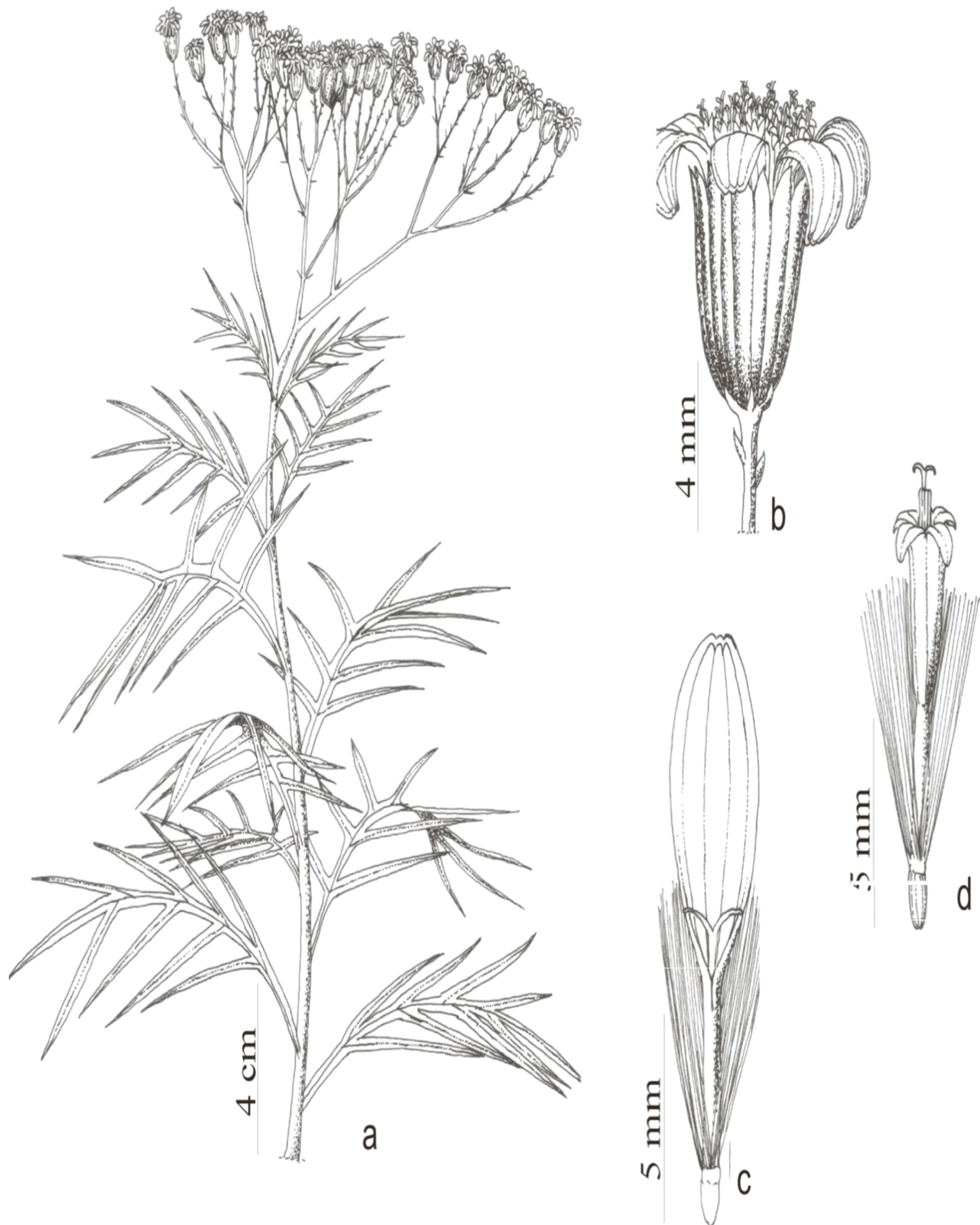
1.1.2.1 *Senecio brasiliensis* (Spreng) Less

Senecio brasiliensis é uma espécie vegetal nativa, não endêmica, pertencente à família Asteraceae e ao gênero *Senecio*, popularmente conhecida como Maria-mole, flor das almas, tasneirinha, cravo-do-campo, e flor-de-finados (Figura 1). É um subarbusto, perene, herbáceo, ereto, e que pode chegar a 2 metros de altura, possui caule liso, cilíndrico, ceríceo, geralmente ramificado na base e parte superior; folhas alternadas, helicoidais, pecioladas, com o limbo profundamente recortado, inflorescência terminal do tipo corimbo com capítulos radiados de coloração amarela, fruto do tipo aquênio, se propaga por meio de sementes (Figura 2) (MOREIRA; BRAGANÇA, 2011; OLIVEIRA et al., 2015; SANDINI et al., 2013).

Figura 1: *Senecio brasiliensis* (Spreng) Less: a) arbusto em período de brotação. b) folhas. c) capítulo d) arbusto em período de floração.

Fonte: Adaptado de <http://www.ufrgs.br/fitoecologia/florars/open_sp.php?img=7433>

Figura 2: Morfologia de *Senecio brasiliensis* (Spreng) Less., a) ramo florido. b) capítulo. c) flor do raio. d) flor do disco



Fonte: Adaptado de Oliveira et al (2015, p. 709).

É uma espécie muito frequente em áreas de vegetação campestre ou degradada, pastagens e terrenos baldios, de fácil propagação, se distribuindo abundantemente em condições ideais de umidade, luz, e temperatura. Durante os meses de maio a agosto a espécie passa pelo seu período de brotação, florescendo entre os meses de outubro a novembro. Geralmente está presente nas regiões Sul, Sudeste e Centro-Oeste do Brasil, além de também ocorrer no Uruguai, Paraguai, e Argentina (BRIGHENT, 2010; SANDINI et al., 2013; TELES, 2015).

Apesar de ser conhecida por sua toxicidade e ser uma das principais causadoras de danos econômicos à pecuária na região Sul do Brasil, é utilizada popularmente para fins medicinais em tratamentos anti-inflamatórios, de feridas, queimaduras, alergia, úlceras gástricas e dores estomacais, porém seu uso indiscriminado pode causar graves danos à saúde humana, e relatos de casos de intoxicação por parte das folhas já foram descritos (MAGNABOSCO et al., 1997; SOUZA, et al., 2015; TANIGUCHI et al., 2002). Toxicidade em insetos já foi reportada, um estudo revelou que extrato aquoso de *S. brasiliensis* diminuiu a ovoposição de *Bemisia tabaci* tipo B em folha de tomate (RIBEIRO et al., 2009) e causou mortalidade e afetou o desenvolvimento de *Microtheca ochroloma* afetando as suas fases larval e pupal (PONCIO, 2010).

Tal toxicidade se deve ao seu metabolismo secundário e síntese de metabólitos tóxicos, principalmente os alcaloides pirrolizidínicos (APs) conhecidos por suas propriedades tóxicas e carcinogênicas (LANGEL; OBER; PELSER, 2011), capazes de causar hepatotoxicidade em bovinos, ovinos, suínos e equinos (SILVA et al., 2006).

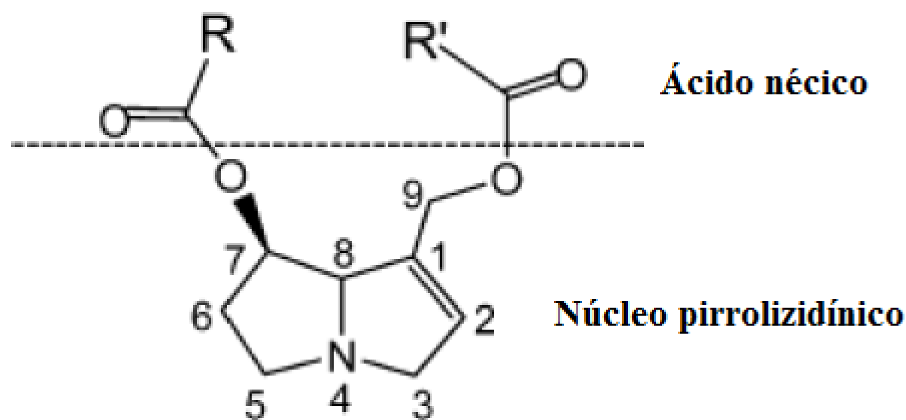
1.1.2.2 Alcaloides pirrolizidínicos e toxicidade

Alcaloides pirrolizidínicos (APs) são metabólitos secundários sintetizados por várias espécies vegetais, encontrados principalmente dentro dos gêneros *Senecio*, *Heliotropium* e *Crotalaria* pertencentes às famílias Asteraceae, Boraginaceae e Leguminosae respectivamente. Porém outras espécies também são capazes de produzir estes metabólitos, estimando-se que cerca de 3% de todas as plantas do mundo sintetizem (PRAKASH et al., 1999; SMITH; CULVENOR, 1981).

Os APs são capazes de apresentar toxicidade aguda ou crônica em virtude da sua estrutura molecular. São moléculas heterocíclicas que contém um núcleo básico de aminoálcool (núcleo pirrolizidínico), e uma porção de ácido alifático (ácido nécico). Como

aspectos fundamentais para a hepatotoxicidade destes metabólitos estão um anel 3-pirrolina; um ou dois grupamentos hidroxilas ligado ao anel pirrolina; um ou dois grupamentos esterificados; e uma cadeia ramificada no resíduo ácido, e como principal característica uma ligação dupla entre os C1 e C2 (Figura 3) (SANDINI et al., 2013; SILVA et al., 2006).

Figura 3: Representação esquemática de um AP.



Fonte: Adaptado de Sandini et al (2013, p. 85).

Nas espécies do gênero *Senecio*, os APs são inicialmente sintetizados nas raízes como N-óxidos de Senecionina e posteriormente transportados para a região superior da planta (folhas e flores), onde então sofrem reações químicas originando diferentes APs (MACEL; VRIELING; KLINKHAMER, 2004), os quais variam estruturalmente, havendo mais do que uma forma conhecida**.** Estes metabólitos distribuem-se pelas flores, folhas, sementes, e caule, havendo variação inter e intraespecífica de concentração, época do ano, e local, no entanto sendo encontrados em maiores quantidades no período de floração (SANDINI et al., 2013; SILVA et al, 2006).

Os APs apresentam toxicidade após a planta ser ingerida e seus constituintes metabolizados no fígado do animal num processo de biotransformação que ocorre em três reações principais: a hidrólise e oxidação, que auxiliam na detoxificação do organismo e a desidrogenação que é responsável pela formação de grupos pirróis altamente tóxicos e instáveis. Essa formação ocorre através da ação das enzimas monooxigenases presentes no retículo endoplasmático (dos hepatócitos) do citocromo P-450 (FU et al., 2002; SANTOS, et al., 2008). Tais derivados pirrólicos possuem capacidade alquilante e se ligam a molécula de DNA, inibindo assim a mitose, levando a uma condição de megalocitose, morte celular por

necrose, diminuição do número de hepatócitos e disfunção hepática (HUAN et al, 1998; SANTOS, et al., 2008). Além disso, estes compostos pirróis podem atingir a circulação geral podendo provocar nefrose e pneumonia intersticial, agravando o quadro e levando o animal a óbito (KARAM; SCHILD; BRAGA, 2011; SANDINI et al., 2013; SILVA et al., 2006).

Entre as principais manifestações patológicas apresentadas após intoxicação por espécies do gênero *Senecio* está a seneciose, ocorrendo principalmente em ruminantes. Esta condição pode suceder após semanas ou meses da ingestão da planta pelo animal, e é a principal causadora de intoxicação e danos econômicos no Rio Grande do Sul. Entre os sintomas estão à perda de peso, e consequente incapacidade e encefalopatia hepática, comprometendo o metabolismo cerebral (KARAM; SCHILD; BRAGA, 2011; SANDINI et al., 2013).

O fato de *Senecio* se alastrar com grande facilidade principalmente em áreas rurais e pastagens, e estar amplamente disponível para consumo dos animais, principalmente no inverno, quando está em seu período de brotação, facilita o consumo das folhas e surgimento de inúmeros casos de intoxicações, tais como a seneciose (KARAM et al., 2004). Assim sendo, as espécies do gênero *Senecio*, principalmente *Senecio brasiliensis*, são consideradas pragas capazes de causar malefícios e grandes perdas econômicas, sendo de suma importância investigações acerca dos potenciais de toxicidade que os seus constituintes possuem e uma melhor compreensão dos mecanismos de ação desta planta nos organismos vivos.

1.1.3 Mecanismos de defesa das células

Frente à toxicidade que muitos compostos apresentam e possíveis danos que podem causar, os organismos vivos sofreram adaptações ao longo do tempo desenvolvendo mecanismos de defesa, com o papel fundamental de manter a homeostase, através de vias que sinalizam para respostas rápidas regulando o metabolismo, desenvolvimento, defesa, reserva energética, sobrevivência e morte celular. Esses mecanismos incluem o sistema de defesa antioxidante bem como suas enzimas, fatores de transcrição, proteínas quinases, proteínas precursoras de apoptose, sistemas que trabalham de forma interligada em prol da homeostase (RAY; HUANG; TSUJI, 2012).

1.1.3.1 Estresse oxidativo e sistema de defesa antioxidante

Radicais livres (RL) são moléculas oxidantes altamente reativas e instáveis, que

possuem meia vida curtíssima e um ou mais elétrons desemparelhados em sua última camada eletrônica (LOBO et al., 2010). Entre estas moléculas encontram-se principalmente às espécies reativas de oxigênio (EROs), destacando-se os radicais superóxido ($O_2^{-\cdot}$) e hidroxila ($OH^{\cdot}$), e o peróxido de hidrogênio (H_2O_2); e as espécies reativas de nitrogênio (ERNs), no qual o peroxinitrito ($ONOO^{-\cdot}$) se sobressai como um dos agentes mais danosos (BARREIROS; DAVID; DAVID, 2006; LOBO et al., 2010).

A presença dos RL é essencial para a manutenção de funções fisiológicas e homeostáticas do organismo, podendo auxiliar na resposta imune contra infecções, sinalização intercelular e na apoptose (BARREIROS; DAVID; DAVID, 2006; SCHNEIDER; OLIVEIRA, 2004). O processo de geração das EROs ocorre naturalmente no organismo, sendo que em torno de 2 a 5% do oxigênio respirado as originam (SCHNEIDER; OLIVEIRA, 2004).

No entanto, quando produzidos em excesso, estes radicais podem promover danos a moléculas essenciais como o DNA, proteínas e lipídeos, podendo acarretar em peroxidação lipídica oxidação proteica e alterações na atividade das enzimas em geral (LOBO et al., 2010; OGA; CAMARGO; BATISTUZZO, 2008). Este desequilíbrio entre a geração dos radicais e a capacidade antioxidante do organismo de combatê-los e/ou eliminá-los do organismo, caracteriza um estado denominado de estresse oxidativo (LOBO et al., 2010; SCHNEIDER; OLIVEIRA, 2004).

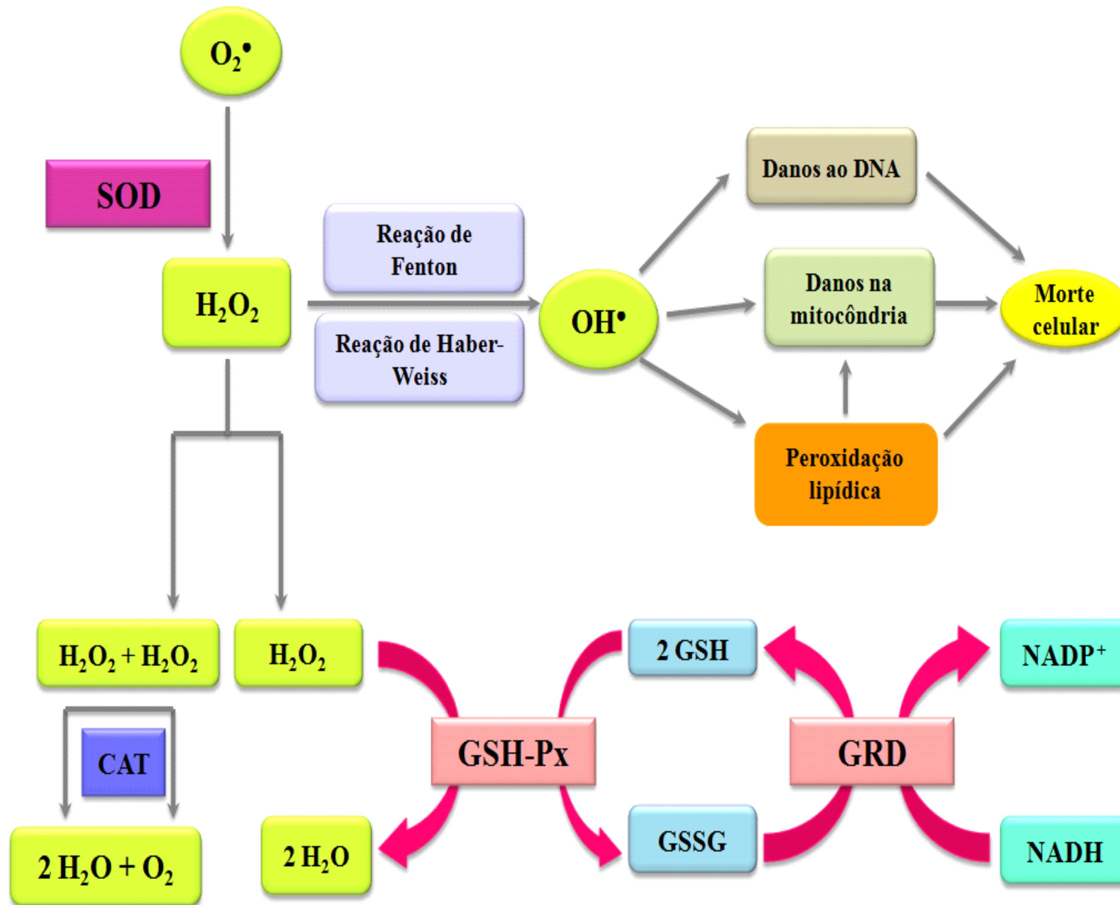
Os antioxidantes são moléculas doadoras de elétrons, que podem inibir a oxidação de outras moléculas, sendo capazes de estabilizar os radicais livres no organismo (PAM-HUY; HE; PAM-HUY, 2008). Eles podem ser obtidos a partir de uma dieta saudável baseada no consumo de vegetais e frutas, capazes de auxiliar no combate a doenças relacionadas com o acúmulo de radicais livres, ou fazerem parte da capacidade endógena do organismo de combater os radicais (PAM-HUY; HE; PAM-HUY, 2008).

O sistema de defesa antioxidante é um mecanismo de proteção e detoxificação, que tem como objetivo impedir ou diminuir os danos causados pelos radicais livres mantendo-os a níveis fisiológicos, através de mecanismos preventivos, varredores e de reparo, impedindo a formação dos radicais, e seu ataque aos componentes celulares, auxiliando na restauração do organismo (BARBOSA et al., 2010; BARREIROS et al., 2006; KOURY; DONANGELO, 2003). Destacam-se neste sistema o tripeptídeo glutationa (GSH), e principalmente as enzimas

antioxidantes glutationa S-transferase (GST), glutationa peroxidade (GPx), glutationa redutase (GR), catalase (CAT) e superóxido dismutase (SOD) (PAM-HUY; HE; PAM-HUY, 2008).

GSH (glutationa reduzida) é o tiol de baixa massa molecular de maior abundância nas células, e um ótimo agente redutor devido a seu radical sulfidrila (SH) que o capacita ser um excelente doador de elétrons (COGO et al., 2009; HUBER; ALMEIDA, 2008). No processo de detoxificação de xenobióticos, a GSH é oxidada a glutationa oxidada (GSSG) pelas enzimas glutationa oxidase (GO) e GPx, a qual é reduzida novamente a GSH através da ação da enzima glutationa redutase (GR). Este ciclo de manutenção redox é muito importante para que a atividade protetora da glutationa seja mantida, e fundamental para o equilíbrio do sistema de defesa enzimático (BARBOSA et al., 2010; COGO et al., 2009; TEKMAN et al., 2008). A enzima GST auxilia na detoxificação do organismo, catalisando a conjugação da GSH a compostos endógenos ou exógenos como xenobióticos, e facilitando a excreção destes (COGO et al., 2009; HUBER; ALMEIDA, 2008). A enzima SOD catalisa a dismutação do radical superóxido ($O_2^{\bullet -}$) em H_2O_2, o qual é normalmente gerado nos organismos aeróbicos, durante o processo de oxidação (COGO et al., 2009). O H_2O_2 formado é então decomposto pela CAT em O_2 e H_2O (COGO et al., 2009), porém pode este H_2O_2 também ser removido através da atividade do sistema GPx (Figura 4) (LOBO et al., 2010; PAM-HUY; HE; PAM-HUY, 2008). No entanto, na falta deste sistema de detoxificação de H_2O_2, pode haver a geração do radical $OH^{\bullet}$, através das reações de Fenton e de Haber-Weiss, este radical é altamente instável e reativo e não há sistema enzimático de defesa (BARBOSA et al., 2010).

Figura 4: Esquema do mecanismo de ação do sistema de defesa antioxidante.



Fonte: Adaptado de Barbosa et al (2010, p. 634) e Morón; Cartilla-Cortázar (2012, p. 90).

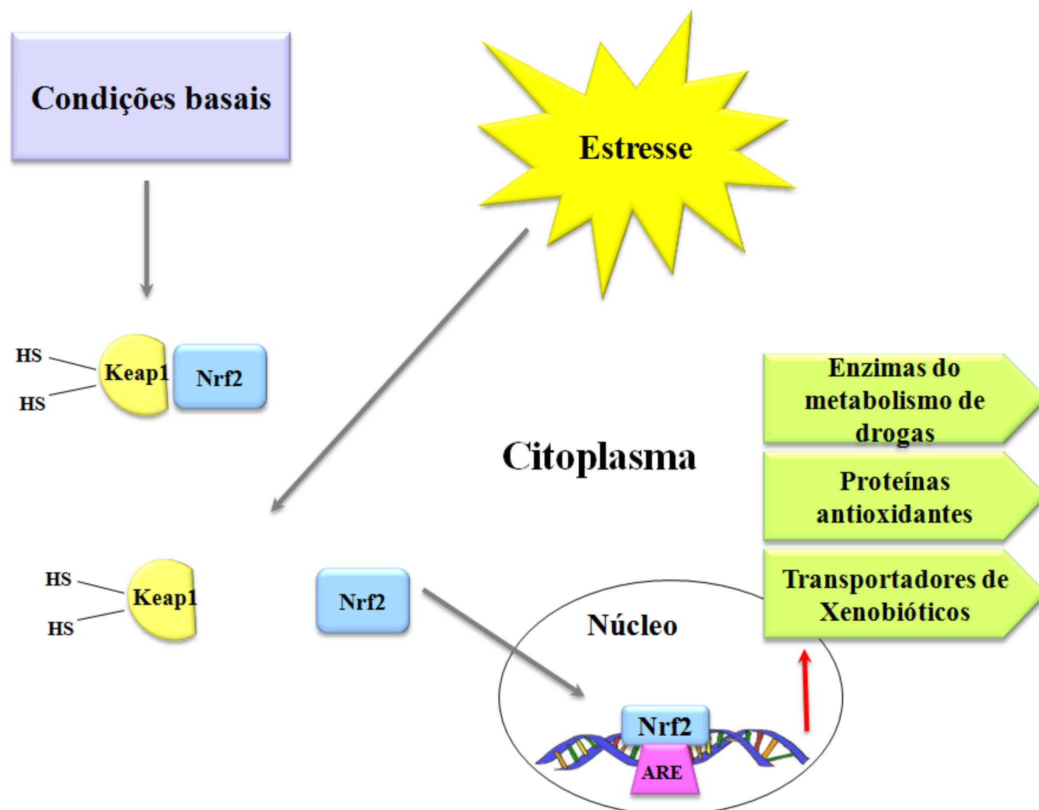
1.1.3.2 Nrf2 e proteínas quinase

Nrf2 (fator nuclear eritróide 2 relacionado ao fator 2) é um fator de transcrição que atua em funções importantes para homeostase, como na diferenciação, proliferação e inflamação**,** além de ser responsável pela regulação do balanço redox celular, e de respostas de detoxificação de fase II, do qual fazem parte as enzimas GST, GPx, do sistema Trx, entre outras envolvidas no metabolismo de xenobióticos eletrofílicos (BRYAN et al., 2013; LOBODA et al., 2016; OSBRURN; KENSLER, 2008). A translocação do Nrf2 para o núcleo é considerada um dos mecanismos de defesa celular mais importante em resposta ao estresse oxidativo, ativando genes que codificam enzimas antioxidantes, em uma tentativa de regulação homeostática (BRYAN et al., 2013; ZHANG, 2006).

Em condições basais, o Nrf2 se mantém no citoplasma formando um complexo com Keap1 (ECH Kelch associando proteína 1), uma proteína rica em cisteína que regula

negativamente o Nrf2, mantendo-o inativo (LOBODA et al., 2016). Porém em condições de estresse, o complexo Nrf2/Keap1 é dissociado, podendo ocorrer uma modificação no grupo SH do keap1 ou fosforilação do Nrf2, facilitando a sua dissociação de keap1, e assim Nrf2 migrar para o núcleo e exercer suas atividades. No núcleo, após se ligar a proteínas Maf, o Nrf2 ativa ARE (elemento de resposta antioxidante) e aumenta transcrição de genes envolvidos com a defesa antioxidante como glutationa peroxidase, glutationa S-transferase, tiorredoxina redutase entre outras (Figura 5) (BRYAN et al., 2013; ZHANG et al., 2013; ZUCKER et al., 2014).

Figura 5: Esquema do mecanismo de ativação do Nrf2.



Fonte: Adaptado de Bryan et al (2013, p. 706).

O estresse oxidativo também é capaz de ativar cascatas de sinalização, como é o caso das proteínas quinases (SON, CAMANDOLA, MATTSON, 2008), que são enzimas fundamentais para a regulação, transdução de sinais e controle intracelular. Elas catalisam a fosforilação de proteínas através da transferência de um grupo fosfato de ATP, ou de GTP, para resíduos de serina, treonina (quinase específica para Ser/Thr) ou resíduos de tirosina (específica para Tyr). A fosforilação destes resíduos exerce papel essencial no controle da

atividade de proteínas (SILVA et al., 2009).

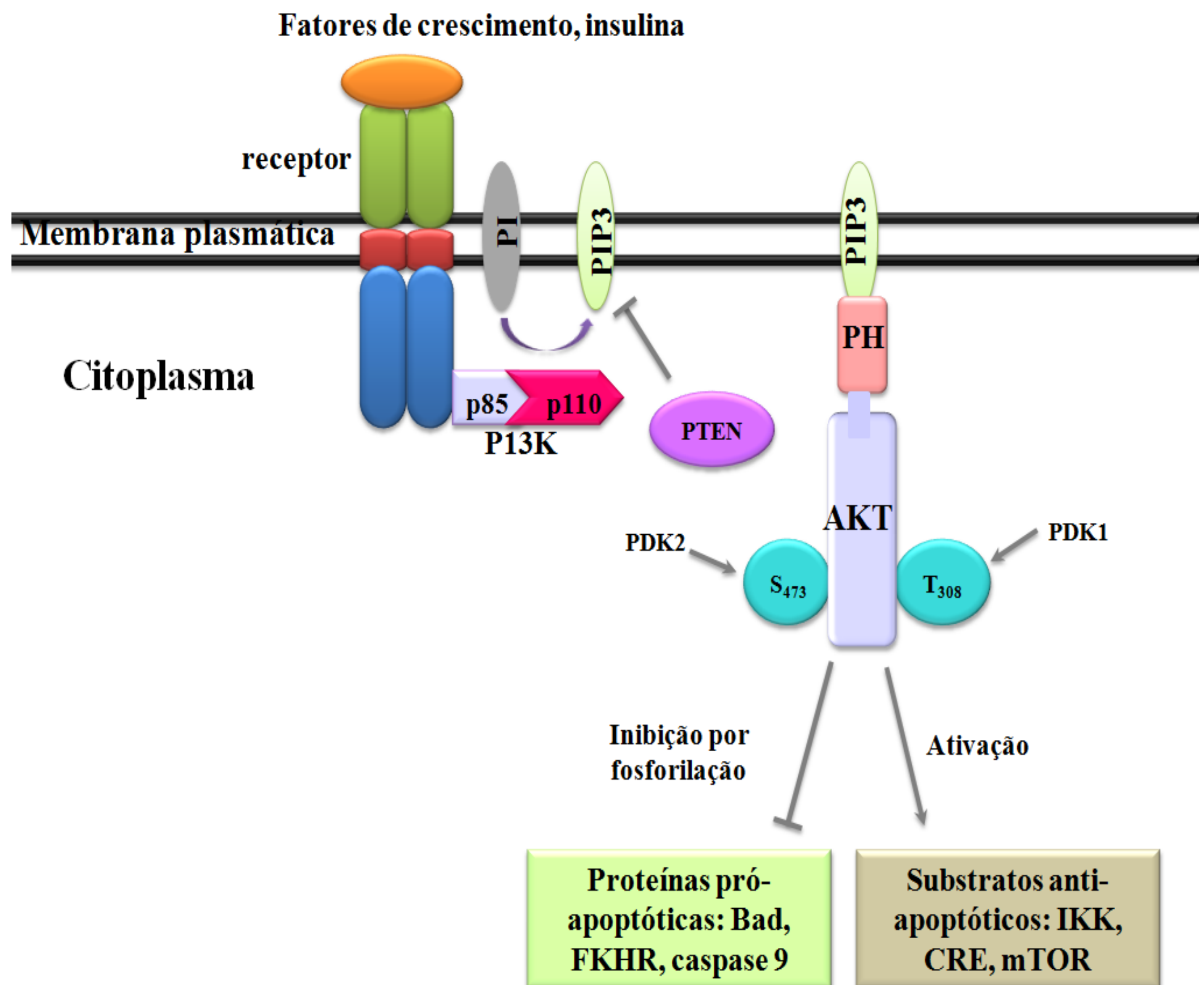
MAPK (proteínas quinases ativadas por mitógenos) compreendem um grupo de proteínas específicas de Ser/Thr que requerem fosforilação desses resíduos para se tornarem ativas. Elas participam de processos de proliferação, expressão gênica, diferenciação, adaptação ao estresse e apoptose. Existem três grupos principais de proteínas quinases, a ERK1/2 (Quinase Regulada por Sinais Extracelulares) relacionada na proliferação e diferenciação celular, e JNK1/2 (c-Jun N-terminal quinase) e a $p38^{MAPK}$ mais envolvidas em processos inflamatórios, respostas a estresse térmico, osmótico e morte celular. Em situações de estresse pode haver ativação por fosforilação ou inibição destas proteínas através de fosfatases (HAYAT, 2013; KENNEDY; CELLURALE; DAVIS, 2007; KIM; CHOI, 2010).

AKT ou também PKB (proteína quinase B) como é conhecida, é uma proteína quinase de resíduos Ser/Thr, que possui um domínio PH (homologia a pleckstrina) na região N-terminal, seguido por um domínio catalítico e uma região regulatória C-terminal (SONG, OUYANG, BAO, 2005). Está envolvida em processos de crescimento e diferenciação celular, progressão do ciclo celular, sobrevivência e inibição da apoptose, além de ser um dos maiores reguladores da sinalização da insulina e metabolismo da glicose (DOWNWARD , 1998). AKT se encontra inativa em células em repouso, porém em resposta a estímulos como fatores de crescimento e insulina é ativada pela via de sinalização da PI3K (fosfatidilinositol 3-quinase), (SONG, OUYANG, BAO, 2005). PI3K é uma proteína formada por duas subunidades: p85 (regulatória) e p110 (catalítica), que se associam após ativação. O receptor do fator de crescimento ativa a subunidade catalítica via recrutamento da subunidade regulatória correspondente ou via ativação de Ras, a qual ativa diretamente a subunidade catalítica. A subunidade catalítica fosforila o PI (fosfatidilinositol) na posição D3 do anel inositol gerando PIP3. A união do PIP3 com o domínio PH da proteína AKT promovem a sua translocação para a membrana plasmática, sofrendo alterações conformacionais que permitem sua fosforilação nos resíduos Treonina e Serina pelas proteínas PDK1 (proteína dependente quinase 1) e PDK2 (proteína dependente quinase 2) respectivamente (Figura 6) (SONG, OUYANG, BAO, 2005).

Um dos seus principais mecanismos após sua ativação é o bloqueio da apoptose. Para isto, AKT utiliza de diferentes mecanismos, podendo inativar a proteína Bad (membro da família Bcl-2), caspase-9, e membros da família Forkhead (FKHR) através de fosforilação. Além disso, ela induz ativação de substratos antiapoptóticos, tais como IKB kinase (IKK) e

CREB (proteína de ligação-elemento de resposta do cAMP) (CHANG, et al., 2003; SONG, OUYANG, BAO, 2005). Também relaciona-se com o crescimento e proliferação celular, através da regulação da quinase mTOR (proteína alvo da rapamicina em mamíferos), que atua em resposta a disponibilidade nutricional e ao estímulo por fatores de crescimento (ZAROGOULIDIS et al, 2014). A via da AKT também é regulada negativamente, podendo ser inibida pela PTEN (fosfatase homóloga a tensina) através da degradação de PIP3, que remove o grupo fosfato na posição D3, levando o PIP3 a sua conformação original. Desta forma, havendo uma significativa redução dos níveis de fosforilação da proteína AKT (GEORGESCU et al 2010).

Figura 6: Esquema da via de sinalização PI3K/AKT.



Fonte: Adaptado de Marques (2010, p. 24).

1.1.4 Apoptose

O controle da proliferação e sobrevivência celular é um mecanismo regulatório essencial para manter o equilíbrio do número populacional de células. Apoptose é um processo de morte celular programada extremamente regulado, totalmente dependente de sinalização celular específica, podendo ocorrer pelas vias intrínseca (intracelular) ou extrínseca (extracelular) (MCILWAIN; BERGER; MAK, 2013).

A via intrínseca é a mais comum e ativada por estresse intracelular em resposta a injúrias, como espécies reativas (ELMORE, 2007). Este tipo de morte é mediada pela via mitocondrial e regulada por proteínas anti-apoptóticas, que constantemente inibem as pró-apoptóticas (ELMORE, 2007). Com a ativação desta via, a ação das proteínas anti-apoptóticas (Bcl2 entre outras) é neutralizada, e pró-apoptóticas (Bax, Bak entre outras) são ativadas contribuindo para a permeabilização da membrana mitocondrial. Moléculas pró-apoptóticas são liberadas após consequente rompimento da membrana mitocondrial, que acarreta na liberação e associação do citocromo C com Apaf-1 (fator de ativação de apoptose 1) afim de ativar pró caspase 9 e formar o apoptossomo (complexo apoptossômico), que ativa a caspase 9, iniciando apoptose pela clivagem e ativação das caspases executoras (ELMORE, 2007; CZABOTAR et al., 2014; MCILWAIN; BERGER; MAK, 2013).

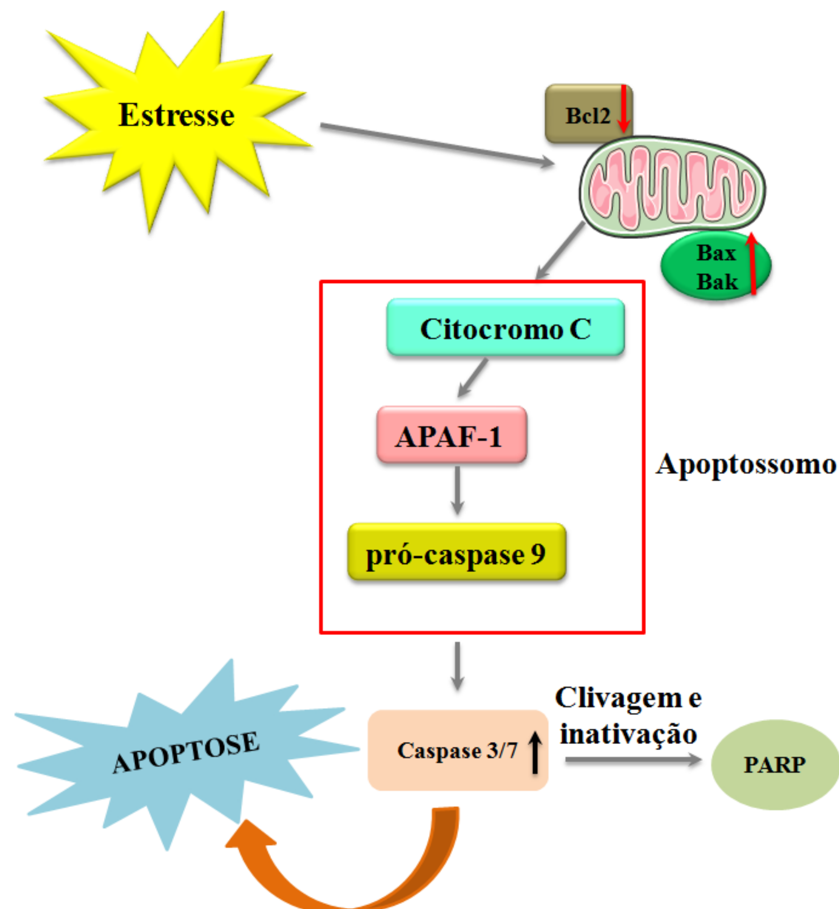
Já na via extrínseca, fatores externos podem reconhecer e se ligar aos receptores de fatores de necrose tumoral (rTNF) presentes na membrana, chamados de receptores de morte celular, iniciando assim uma resposta de sinalização celular e ativando a cascata das caspases (ELMORE, 2007; MCILWAIN; BERGER; MAK, 2013).

Proteínas caspases (proteases aspartato-específicas dependentes de cisteína) são uma família de proteases que possuem uma cisteína em seu sítio ativo e a utilizam como grupo nucleofílico na clivagem de substratos, e desempenham papel fundamental no processo de morte celular programada. São sintetizadas na forma de precursores inativos como pró-caspases e são ativadas em caspases quando deflagrado o processo de morte celular. Sete caspases estão envolvidas no processo de apoptose, sendo elas iniciadoras (caspases 2,8,9 e 10) que podem ser autoclivadas e ativar caspases efetoras ou executoras (3,6 e 7) em mamíferos (ELMORE, 2007; MCILWAIN; BERGER; MAK, 2013). Caspase 3 é capaz de inativar proteínas que atuam para a sobrevivência celular, como é o caso da proteína Poli (ADP-Ribose) Polimerase conhecida como PARP, uma família de proteínas que exercem

importantes funções para a homeostase celular, detecção e reparo de danos ao DNA, expressão gênica, e apoptose, auxiliando assim na manutenção da integridade do genoma (MORALES et al., 2014).

A isoforma PARP-1 é um dos principais membros da família, e uma enzima nuclear que é ativada em reposta a danos no DNA, com a capacidade de poli-ADP-ribosilação, um mecanismo que envolve a transferência de unidades de ADP-ribose para proteínas nucleares utilizando NAD^+ (nicotinamida acetil dinucleotídeo) como substrato, o que leva a formação de polímeros de ADP-ribose essenciais para manter a integridade do genoma (MORALES et al., 2014; SCHREIBER et al., 2006). Em condições de homeostase, PARP-1 encontra-se em sua forma inativa no núcleo celular, centrossomos, e mitocôndrias. No processo de apoptose PARP-1 é transportada para o citoplasma, onde serve de substrato para as proteínas caspases 3/7 sendo clivada e inativada por elas (Figura 7) (MORALES et al., 2014; SCHREIBER et al., 2006).

Figura 7: Esquema da via intrínseca de apoptose, ativação de caspases e clivagem de PARP.



Fonte: Adaptado de BERKI et al (2011, p. 124).

1.1.5 Regulação do metabolismo energético

Além destes mecanismos de defesa, o metabolismo energético e a regulação de sua complexidade são essenciais para a manutenção da homeostase e sobrevivência dos organismos. A glicose é imprescindível para este processo, como a principal fonte de energia, podendo ser armazenada na forma de amido e glicogênio (NELSON; COX, 2014).

Os mecanismos de sinalização e regulação transcricional do metabolismo dos carboidratos, proteínas, e lipídeos, possui similaridade entre humanos e *Drosophila melanogaster* (SHINGLETON, 2010), uma vez que Drosophila possui peptídeos semelhantes à insulina conhecidos como Dilps (peptídeos similares a insulina de Drosophila) que detém similaridades estruturais e funcionais com o fator de crescimento semelhante à insulina tipo 1 (IGF-1), insulina, e com a via de sinalização IIS (insulina/IGF-1) de vertebrados. Dilps possuem papéis essenciais na regulação da homeostase da glicose, atuando na manutenção dos seus níveis de hemolinfa na mosca, e na regulação de lipídios, reprodução, crescimento, resistência ao estresse e envelhecimento (BIRSE et al., 2011; HASELTON; FRIDELL, 2010).

Sete diferentes Dilps são encontradas no genoma de Drosophila, denominadas Dilp1, Dilp2, Dilp3, Dilp4, Dilp5, Dilp6 e Dilp7, e encontrando-se expressas em uma variedade de tecidos incluindo cordão nervo ventral, glândulas salivares, ovários e intestino larvais, e cérebro larval e adulto. As Dilps 1-5 possuem maior similaridade com a insulina de mamíferos, enquanto a Dilp6 possui mais similaridade com IGF-1 (HASELTON; FRIDELL, 2010; SHINGLETON, 2010; ZHANG et al., 2009).

1.1.6 *Drosophila melanogaster*

O desenvolvimento de metodologias alternativas utilizando insetos como organismo modelo para estudos de toxicologia vem ganhando destaque na comunidade científica, principalmente devido há grande tendência na redução do sacrifício de animais (MORALES, 2008). Além disso, o crescente aumento de pesquisas relacionadas com a utilização de produtos naturais, principalmente de origem vegetal, bem como da exposição a xenobióticos, evidencia a necessidade de modelos alternativos para ensaios toxicológicos iniciais. Os insetos apresentam vantagens únicas, como uma resposta semelhante a roedores em relação à exposição a contaminantes, além de possuírem fácil manuseio e observação, rápida proliferação e crescimento, e sem necessitar de aprovação de comitês de ética para a sua

utilização (SCULLY; BIDOCHKA, 2006).

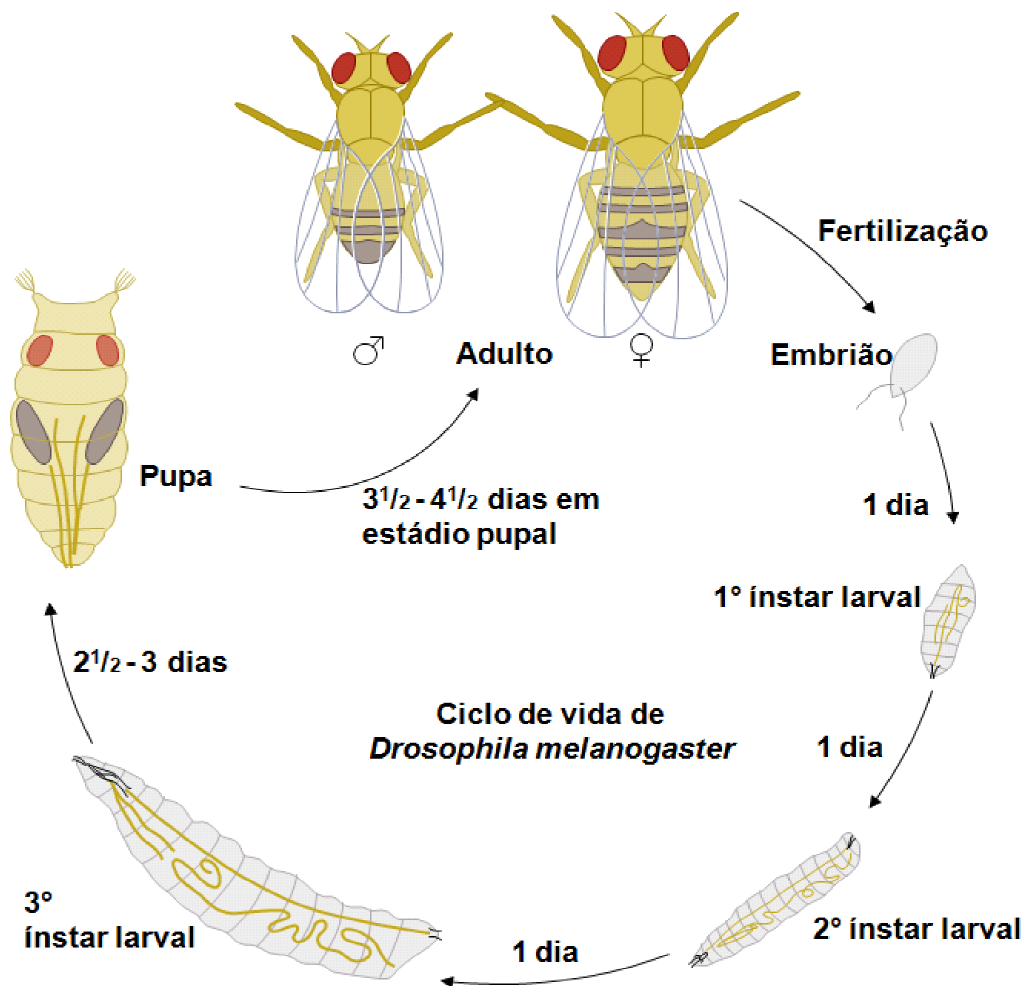
Dentre os modelos alternativos a mosca da fruta, *Drosophila melanogaster* (Figura 8) é um invertebrado pertencente à ordem Diptera e a família Drosiphilidae, e de larga utilização em estudos de bioquímica, toxicologia, genética, biologia molecular, bem como para *screening* de compostos e análise de ações biológicas de substâncias naturais (STRANGE, 2016).

Figura 8: Modelo experimental *Drosophila melanogaster*

Fonte: <http://www.yourgenome.org/stories/fruit-flies-in-the-laboratory>

Entre as suas principais vantagens estão o rápido ciclo de vida (Figura 9), manutenção de baixo custo, tamanho reduzido, dimorfismo sexual aparente, fácil manipulação, falta de imposições éticas para seu uso e principalmente por possuir alta homologia com genes envolvidos em doenças em humanos (PANDEY; NICHOLS, 2011; STRANGE, 2016). Muitos mecanismos biológicos relacionados ao controle desenvolvimental e sobreviência, são conservados entre Drosophila e humanos, contribuindo assim para o seu uso em pesquisas que busquem avaliar a exposição de determinadas substâncias durante as etapas de desenvolvimento (JENNINGS, 2011).

Figura 9: Ciclo de vida de *Drosophila melanogaster*



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2. Justificativa

Estudos acerca dos potenciais que a flora brasileira apresenta se fazem cada vez mais necessários principalmente devido ao uso popular indiscriminado das plantas para fins terapêuticos e ausência de embasamentos científicos a respeito da toxicidade e possíveis interações que as mesmas podem possuir com os organismos. O conhecimento acerca dos alvos moleculares modulados por constituintes de extratos vegetais permite que estas espécies sejam utilizadas com abordagem terapêutica em uma variedade de patologias onde tais alvos estejam implicados bem como no surgimento de produtos venham a contribuir com o bem estar da sociedade como agentes inseticidas. Desta forma nosso estudo contribui com a

descrição de alvos moleculares implicados no desenvolvimento e homeostasia da *Drosophila melanogaster*, buscando contribuir com a compreensão dos mecanismos envolvidos na toxicidade de *Senecio brasiliensis* e aplicabilidade de seu extrato.

3. Objetivos

3.1 Objetivo geral

Investigar a toxicidade da exposição do extrato hidroalcoólico das folhas de *Senecio brasiliensis* (Spreng) Less., (EHSB) no modelo experimental de *Drosophila melanogaster* durante a fase adulta e larval. Na fase larval compreender e elucidar a toxicidade e os mecanismos bioquímicos, moleculares e de transdução de sinais envolvidos na ação desta planta.

3.2 Objetivos específicos

- Realizar a caracterização fitoquímica do EHSB quanto à presença de compostos fenólicos e flavonóides;
- Avaliar a atividade antioxidante *in vitro* do EHSB;
- Avaliar a viabilidade e desempenho locomotor de *D. melanogaster* adultas expostas a EHSB;
- Avaliar a taxa de *D. melanogaster* adultas eclodidas após exposição ao EHSB durante o seu período embrionário, larval e pupal;
- Analisar a atividade das enzimas antioxidantes catalase, glutationa S-Transferase e superóxido dismutase em larvas de 3° ínstar de *D. melanogaster* expostas ao EHSB;
- Analisar a viabilidade celular e a razão de glutationa (GSH/GSSG) em larvas de 3° ínstar de *D. melanogaster* expostas a ao EHSB;
- Determinar a produção de espécies reativas de oxigênio em larvas de 3° ínstar de *D. melanogaster* expostas ao EHSB;
- Analisar a modulação/fosforilação das proteínas quinases ERK1/2, JNK1/2, $p38^{MAPK}$, AKT, e clivagem de PARP em larvas de 3° ínstar de *D. melanogaster* expostas ao EHSB;
- Analisar a atividade de caspase 3/7 em larvas de 3° ínstar de *D. melanogaster* expostas ao EHSB;
- Analisar a presença ou ausência de danos a nível transcricional dos genes GPDH,

CAT, SOD, Nrf2, DRICE, TrxR, Dilp2, Dilp3, Dilp5 e Dilp6 em larvas de 3° ínstar de *D. melanogaster* expostas ao EHSB.

4. Resultados

Os resultados que fazem parte desta dissertação estão apresentados sob a forma de manuscrito. Os itens Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas, encontram-se no manuscrito, os quais estão dispostos na forma em que foram submetidos para publicação em Comparative Biochemistry and Physiology, part C ISSN: 1532-0456.

Senecio brasiliensis impairs eclosion rate and induces apoptotic cell death in larvae of *Drosophila melanogaster*

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Abstract

Senecio brasilienis (Spreng) Less., is a species native from Brazil, popularly known as "maria mole", and known to induce hepatotoxicity due to its high content of Pyrrolizidine alkaloids. Despite its toxicity, this plant is widely used in Brazilian folk medicine. Considering the antagonizing effects described for *S. brasiliensis,* we describe here molecular markers involved in the toxicity of hydroalcoholic extract from leaves of *S. brasiliensis* (HESB) in *Drosophila melanogaster.* Phytochemical analysis of HESB revealed the presence of phenolic acids and flavonoids. A significant antioxidant potential against $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical was found in parallel. Ingestion of extract did not alter the survival and locomotor activity of adult flies. However when ingested along the larval developmental phase, the eclosion rate of flies was interrupted at higher concentration of extract. To comprehend this phenomenon several analysis were conducted in larvae. HESB stimulated activity of antioxidant enzymes SOD and GST, and increased GSH/GSSG ratio and ROS production. Additionally, HESB caused a significant decrease of cell viability. The mRNA expression of Nrf2, TrxR, CAT, Drice and Dilp6 were also significantly up-regulated. HESB caused significant decrease on the phosphorylation of MAPKs and AKT. In parallel, PARP cleavage and caspases 3/7 activity were stimulated. In addition, glucose, glycogen and triglycerides levels were decreased. Taken together our study depicts a disruption in the eclosion of *D. melanogaster* possibly attributed to the inhibition of kinases implied in developmental process, energetic demand and induction of apoptotic cell death process.

Keywords: caspases; MAPK; maria-mole; Nrf2; oxidative stress; toxicity.

1. Introduction

Several plant species are used indiscriminately by human population in the form of teas and infusions with therapeutic purposes. However the consumption of those species may cause toxic effects (Sen and Samanta, 2015). The vegetal secondary metabolism comprises a variety of metabolites that evolved in order to promote the plants survival providing protection against general stresses, as environmental factors, insects, herbivores, predators, pathogens and UV radiation. Some of this substances are restricted to certain species constituting potential source of bioactive compounds useful in scientific research contributing for the discovery of new products and medicines (Cespedes et al., 2015; Mierziak et al., 2014; Mishra and Tiwari, 2011; Tiwari and Rana, 2015).

Senecio brasiliensis (Spreng) Less., popularly known as “maria mole” belongs to *Senecio* genus and Asteraceae family, is a native plant from Brazil, found usually in south and southeast of the country (Toma et al., 2004). Many plants, mainly within the *Senecio* genus are able to synthesize hepatototoxic metabolites known as Pyrrolizidine alkaloids (PAs) (Sandini et al., 2015). *S. brasiliensis* is one of the most toxic species of the genus, and due to presence of these alkaloids is capable to cause hepatotoxicity and poisoning in horses and cattle and lead them to death (Karam et al., 2011). Despite this toxicity, same parts of the plant including leaves and flowers are used in Brazilian folk medicine as an anti-inflammatory, gastric ulcers and stomach pain treatments (Sandini et al., 2015; Toma et al., 2004).

A variety of biological properties were described to *Senecio* genus, such as antiulcerogenic and anti-inflammatory for *Senecio brasiliensis* (De Souza et al., 2015; Toma et al., 2004); anti-inflammatory *for Senecio salignus* and *Senecio flammeus* (Pérez González et al., 2013; Xiao et al., 2014); antibacterial for *Senecio tenuifolius* (Manubolu et al., 2013); antiplasmodial for *Senecio smithioides* (Mollinedo et al., 2015); antioxidant and cytotoxic for *Senecio graciliflorus* (Lone et al., 2014); cytotoxic for *Senecio delphinifolius* (Tidjani et al., 2013); antioxidant, hemolytic and cytotoxic for *Senecio nutants* (Lizarraga et al., 2012).

Free radicals can be classified in reactive oxygen species (ROS) and reactive nitrogen species (RNS), and are produced by living organisms in normal cellular metabolism contributing for physiological homeostasis (Birben et al., 2012; Pisoschi and Pop, 2015). Oxidative stress is a condition caused by overproduction of free radicals and/or alterations in antioxidant defense system of the organism caused by environmental stressors or pathological conditions (Pisoschi and Pop, 2015; Birben et al., 2012). Physiological mechanisms are responsible for protecting cells from damage caused by oxidative stress, including the enzymatic [i.e. glutathione-S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD)] and nonenzymatic (i.e. glutathione GSH) antioxidant system (Birben et al., 2012).

The signaling pathways of the Nrf2 transcription factor (nuclear factor erythroid 2 like 2) protein kinases are also important part of this mechanism (Bryan et al., 2013). The Nrf2 is a transcription factor that regulates expression of genes involved in antioxidant and detoxification response, and is one of the most important defensive signaling pathways in animals (Bryan et al., 2013; Chen et al., 2015). The protein kinases such as MAPK (mitogen-activated protein kinase) and PI3K/AKT are implied in many cellular functions, such as

growth, proliferation, differentiation, survival and apoptosis (Bryan et al., 2013; Kim and Choi, 2010; Son et al., 2009), the modulation of these targets by botanical compounds is scarcely demonstrated and its implication still unknown.

The energetic metabolism is also an essential part to maintenance of homeostasis and survival of the living organisms, and its regulation is attributed to insulin/IGF1 signaling (IIS) pathway (Grönke et al., 2010). Those peptides are also related to growth, proliferation and longevity processes in response to metabolic adaptation, feeding changes and environmental stresses (Chatterjee et al., 2014). In *Drosophila melanogaster*, Dilps (Drosophila insulin-like peptides) have important functions as regulation of growth, glucose homeostasis, maintenance of hemolymph, storage lipids, reproduction, stress resistance, and aging in the fruit fly (Birse et al., 2011; Haselton and Fridell, 2010).

Considering the antagonizing effects described to *Senecio brasiliensis* and the use of this plant for therapeutic purposes by the population, it is necessary to better understand about its chemical components, toxicity and interaction with living organisms. In this aspect *Drosophila melanogaster* known as fruit fly is a powerful and advantageous model for elucidation of embryonic development, behavior, and aging, sharing with mammals important mechanism and pathways implied in such process (Jennings, 2011).

In this study we aimed to investigate the biological effects of the hydroalcoholic extract of leaves of *Senecio brasiliensis* (HESB) exposure in adult and larval phases of *Drosophila melanogaster* taking into account the modulation of oxidative stress and antioxidant defense markers, cell death and general development.

2. Material and Methods

2.1 Materials

Sucrose (S5016); Reduced glutathione (GSH, G4251); tetramethylethylenediamine (TEMED, T9281); Quercetin (Q4951); 5,5-dithiobis (2-nitrobenzoic acid) (DTNB, D8130); 1-Chloro, 2,4-dinitrobenzene (CDNB, 237329); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, M2128); 2',7'-dichlorofluorescein diacetate (DCF-DA, 35845); Resazurin sodium salt (R7017); D- Mannitol (M9647); K_2KO_4P (1110216); KH_2PO_4 (P0662); Hepes Minimum 99,5% (Titration, H3375); Albumin from bovine serum (BSA, A6003); Triton X-100 (T8532); Agar (A1296); β-mercaptoethanol (M6250); anti-rabbit

immunoglobulin (HRP peroxidase-linked antibody) were obtained from Sigma-Aldrich (São Paulo, SP, Brazil). Anti-phospho-p38 (Thr180/Tyr182); anti-phospho JNK1/2 (Thr183/Tyr185); anti-phospho ERK1/2 (Thr202/Tyr204) and anti-total-ERK1/2, anti-phospho AKT (Ser473) and β-actin antibodies were purchased from Cell Signaling Technology (Danvers, MA). SDS, acrylamide, bis-acrylamide, hybond nitrocellulose were obtained from GE Healthcare Life Division (Uppsala, Sweden). Poly(ADP)-ribose polymerase (PARP) antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). DNAse I Amplification Grade (18068-015) from Invitrogen by Life Technologies; SYBR Select Master Mix (4472908) from Biosystems by Life Technologies; TRIzol (15596026) from Ambion/RNA by Life Technologies; and iScript cDNA Synthesis kit (170-8891) was obtained from Biorad. Caspase-Glo 3/7 (G7790) was obtained from Promega. All other chemicals and reagents used here were of the highest analytical grade.

2.2 Plant material and extract preparation

The leaves *S. brasiliensis* were collected in the municipality of São Gabriel, Rio Grande do Sul, Brazil (30°19'47.5"S 54°21'49.7"W) in November 2014. The plant material was identified at the Herbarium of the Federal University of Pampa – UNIPAMPA, where a voucher specimen was deposited (HBEI 1437). The fresh leaves *S. brasiliensis* (526 g) was washed under running water and air dried. The air dried materials were macerated with 99.9% ethanol and water (1:1, v/v), for seven days. The mixture was filtered and the solvent was evaporated and lyophilized to obtain 22 g of hydroalcoholic extract of leaves of *S. brasiliensis* (HESB; crude extract) (Matos, 1997).

2.3 Qualitative and quantitative analyses of phenolics and flavonoids compounds by HPLC-DAD

HESB at a concentration of 15 mg/mL was injected by means of a model SIL-20A Shimadzu Auto sampler. Separations were carried out using Phenomenex C_{18} column (4.6 mm x 250 mm x 5 mm particle size). The mobile phase was solvent A = water:acetic acid (98:2, v/v) and solvent B = acetonitrile. The gradient program was started with 95% of A and 5% of B until 2 min and changed to obtain 25%, 40%, 50%, 60%, 70% and 80% B at 10, 20, 30, 40, 50 and 80 min, respectively, following the method described by Boligon et al., (2015). The sample and mobile phase were filtered through 0.45 μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were

prepared in the HPLC mobile phase at a concentration range of 0.025 – 0.500 mg/mL. Quantifications were carried out by integration of the peaks using the external standard method, at 254 nm for gallic acid; 327 nm for chlorogenic, rosmarinic and caffeic acids; and 366 for quercetin, and vitexin. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 600 nm). Calibration curve for gallic acid: $Y = 12538x + 1197.3$ ($r = 0.9998$); chlorogenic acid: $Y = 13257x + 1265.8$ ($r = 0.9999$); caffeic acid: $Y = 12584x + 1237.6$ ($r = 0.9996$); rosmarinic acid: $Y = 11874x + 1345.7$ ($r = 0.9997$); quercetin: $Y = 11495x + 1185.7$ ($r = 0.9998$); and vitexin: $Y = 13706x + 1278.9$ ($r = 0.9999$). All chromatography operations were carried out at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves, as defined by Brito et al., (2015). LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

2.4 In vitro antioxidant activity determination

All spectrophotometric assay of the analysis of *in vitro* antioxidant properties were performed in 96-well plates using the EnSpire® multimode plate reader (PerkinElmer, Waltham, MA).

2.4.1 ABTS radical scavenging assay

The antioxidant activity of HESB in the reaction with $ABTS^{\bullet+}$radical was determined according to the method of Baltrušaityte et al., (2007) with some modifications. $ABTS^{\bullet+}$ radical solution was generated by oxidation of solutions prepared of 1mL of 7mM 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt stock solution with 17.5 µL of 140mM potassium persulphate ($K_2S_2O_8$). The mixture was left to stand in the dark at room temperature for 12–16 h before use. For the evaluation of antioxidant capacity, the ABTS solution was diluted with distilled water to obtain the absorbance of 0.700 ± 0.020 at 734 nm. Two hundred µL of ABTS solution was mixed with 10 µL of HESB (200 µg/mL) in a microplate and the decrease in the absorbance was measured after 10 min. Ascorbic acid (1 mM) was used as a positive control. The results were expressed as mg of ascorbic acid equivalents (AAEs) per 100 mg HESB (Piljac-Žegarac et al., 2009).

2.4.2 DPPH˙ Radical Scavenging Assay

The scavenging activity towards 2,2-diphenyl-1 picrylhydrazyl ($DPPH^{\bullet}$) radical was evaluated according to the method of Baltrušaityte et al., (2007) with minor modifications. In the presence of an antioxidant, the purple colour of DPPH fades and the change of absorbance can be followed spectrophotometricallyv at 515 nm. In brief, 100 µL of DPPH (300 µM) diluted in ethanol was mixed with 20 µL of HESB (200 µg/mL) in a 96 wells microtitre plate. The final volume of each well was adjusted to 300 µL with ethanol. Ascorbic acid (1 mM) was used as a positive control. The absorbance was determined at 517nm after 45 min incubation. The results were expressed as mg of ascorbic acid equivalents (AAEs) per 100 mg HESB (Piljac-Žegarac et al., 2009).

2.4.3 Total Phenolics

Phenolic compounds from HESB samples were detected by the Folin-Ciocalteu method with minor modifications (Cruz et al., 2014). HESB (200 µg/mL) was mixed with 35 µL 1N Folin-Ciocalteu"s reagent. After 3min, 70 µL 15% Na_2CO_3 solution was added to the mixture and adjusted to 284 µL with distilled water. The reaction was kept in the dark for 2h, after which the absorbance was read at 760 nm. Gallic acid was used as standard (10–400 µg/mL). The results were expressed as g of gallic acid equivalents (GAEs) per 100 g HESB.

2.4.4 Ferric Reducing Antioxidant Power (FRAP)

The reducing capacity of HESB was assayed with the original method of Benzie and Strain, (1996) adjusted to analysis of extract samples. 9 µL of HESB (200 µg/mL) was mixed with of 270 µL of freshly prepared FRAP reagent. The FRAP reagent was prepared by mixing 2.5mL of 0.3 M acetate buffer pH 3.6 with 250 µL of 10mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution and 250 µL of $FeCl_3 \cdot 6H_2O$. The mixture was shaken and left in a water bath for 30min and the absorbance readings were taken at 595 nm. Ammonium iron (II) sulfate hexahydrate was used to calculate the standard curve (100–2000 µM).The reducing ability of extract was expressed as µM of Fe (II) equivalent/100 g HESB (Cruz et al., 2014).

2.5 Fly culture maintenance

Drosophila melanogaster (Harwich strain) was obtained from the National Species Stock Center, Bowling Green, OH, USA. The flies were maintained in incubators at 25 ± 1°C,

12h dark-light photoperiod and 60–70% relative humidity. The basic cornmeal diet was composed of cereal flour, cornflour, water, antifungal agent (Nipagin) and supplemented with dried yeast.

2.6 Senecio brasiliensis exposure

Effect of exposure of HESB during the development and adult life of *Drosophila melanogaster* was observed.

2.6.1 Measure of locomotor activity of flies exposed to HESB

Twenty adult female flies (1-3 days) per group were kept in glass tubes containing cornmeal culture medium with each treatment solution for 7 days. The experimental groups were: Control (cornmeal culture medium only), HESB (1, 5, 10, 50, 100 and 200 mg/mL diluted in cornmeal culture medium). After the period of 7 days, mortality and behavioral tests were quantified. The number of live and dead flies was counted every 24 h and LC_{50} flies were determined. Results were analyzed and plotted as the percentage of fly survival in relation to control. All the experiments were repeated in triplicate.

Behavioral test was determined using the individual negative geotaxis assay. After treatment of 7 days, 10 flies per condition were immobilized on ice for 1–2 min and were gently tapped to the bottom of a glass column and the time that each fly reach of top (5 cm of the column) was counted as described by Jimenez-Del-Rio et al., (2010). The procedure was repeated three times with an interval of 30 seconds. Data represent an average of three replicates per treatment.

2.6.2 Measurement of larval development

Fifty eggs of *Drosophila melanogaster* were transferred to agar medium culture composed of 1.5% agar, 3% sucrose, 1.25% dried yeast, 0.1% Nipagin and distilled water. The group control was agar medium only, and the treatments were HESB at concentrations of 0.01, 0.1, 0.5 and 1 mg/mL dilute in the medium. The number of hatched eggs, larvae, pupae and ecloded flies were counted daily for a period of 20 days, to obtain the hatching curve. Results were analyzed and plotted as ecloded flies of a total 50 eggs. All the experiments were performed in triplicate. Biochemical analysis was performed in third instar larvae, the phase before pupation.

2.7 Sample preparation

Third instar larvae was treated with HESB at 1 mg/mL. For this, eggs were transferred to agar culture medium containing 0 or 1 mg/mL of HESB and after 5 days the third instar larvae were collected. For sample preparation, larvae were removed from the treatment and approximately 2-3 mg/mL of protein was taken as standard (approximately 200 larvae per group) for all assays except for qRT-PCR analysis, where 20 larvae per group were used in a 0.1 mg/mL concentration.

2.8 Protein quantification

The concentration of protein in samples was performed by the method of Bradford, (1976) using bovine serum albumin as the standard. Protein concentration in Western Blotting sample was measured by methodology of Peterson, (1977).

2.9 Cellular viability

Mitochondrial activity was measured by two tests: MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay (colorimetric) and Resazurin assay (fluorescence). For MTT assay, groups of larvae (containing 2-3 mg of protein) were homogenized in 500 µL of isolation medium [220 mM mannitol, 68 mM sucrose, 10 mM KCl, 10 mM HEPES, 0,1% serum albumin (BSA)] and then centrifuged at 1,000 g for 10 min at 4°C. The obtained supernatant was isolated and incubated for 60 min (37°C) in MTT reagent, after the sample was centrifuged and MTT was removed and the sample was incubated in DMSO for 30 min (37°C), as previously described by Rodrigues et al., (2015) with same modifications. When viable, mitochondria convert the MTT to a colorful formazan. The absorbance was read in EnsPire® multimode plate reader (PerkinElmer, Waltham, MA) at 540nm.

Resazurin assay is based on the ability of viable mitochondria to convert resazurin into a fluorescent end product (resorufin) (Rodrigues et al., 2015). No viable samples rapidly lose metabolic capacity and thus do not generate a fluorescent signal (O'Brien et al., 2000). Homogenate preparation followed the same methodology and the supernatant was incubated in the Elisa plates with mitochondrial isolation buffer (without BSA) and resazurin. The fluorescence was monitored at regular intervals of 1 h up to 3h at 544 nm_{ex}/590 nm_{em} using EnsPire® multimode plate reader (PerkinElmer, Waltham, MA). The results were calculated as

a percentage in relation to the control group and values were normalized by protein concentration.

2.10 DCF-DA oxidation assay

2’-7’-Dichlorofluorescein diacetate (DCF-DA) oxidation was used as a general index of ROS as described by Pérez-Severiano et al., (2004). For DCF-DA assay we utilized the same sample for cell viability tests. The supernatant was incubated in the Elisa plates with mitochondrial isolation buffer (without BSA) and DCF-DA reagent. The fluorescence emission of DCF resulting from DCFDA oxidation was monitored at regular intervals at an excitation wavelength of 485nm and an emission wavelength of 530nm in an EnsPire® multimode plate reader (PerkinElmer, Waltham, MA). The rate of DCF formation was calculated as a percentage of the DCF formation in relation to the control group and values were normalized by protein concentration.

2.11 Enzyme assays

Groups of larvae were homogenized in 500 µL of 20 mM HEPES buffer, pH 7.0, and centrifuged at 20,000 g for 30 min at 4°C. The supernatant was used for determination of glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) according to methods described earlier. GST (EC 2.5.1.18) activity is based on formation of the conjugated complex of CDNB and GSH at 340 nm. The reaction was conducted in a mix consisting of 100 mM phosphate buffer pH 7.0, 1 mM EDTA, 1 mM GSH and 2.5 mM CDNB according Habig and Jakoby, (1981). CAT (EC 1.11.1.6) activity was assayed following the clearance of H_2O_2 at 240 nm in a reaction media containing 50 mM phosphate buffer pH 7.0, 0.5 mM EDTA, 10 mM H_2O_2, 0.012% Triton X100 according to the procedure of Aebi, (1984). SOD (EC 1.15.1.1) activity consists in the inhibition of superoxide-driven oxidation of quercetin by SOD at 406 nm. The complete reaction system consisted of 25 mM phosphate buffer, pH 10, 0.25 mM EDTA, 0.8 mM TEMED and 0.05 µM quercetin, following Kostyuk and Potapovich, (1989). All enzyme activities were performed at room temperature (25±1°C) using a 18 cell holder Agilent Cary 60 UV–Vis® spectrophotometer coupled to a peltier controlled water bath system (Santa Clara, CA).

2.12 Determination of glutathione levels

Reduced (GSH) and oxidized glutathione (GSSG) levels were determined according

Hissin and Hilf, (1976). Groups of larvae were homogenized in 750 μL of 100 mM NaH_2PO_4 buffer pH 8.0 containing 5 mM EDTA, after was added 250 μL of Phosphoric acid, and the samples were centrifuged at 100,000 g for 30 min at 4° C. For measurement of GSH levels, aliquots (10 μL) of the supernatant were mixed with the same homogenization buffer (180 μL). Ten microliters of *O*-phthalaldehyde (1 mg/mL) was added and fluorescence was measured 15 min later using an excitation wavelength of 350 nm and an emission wavelength of 420 nm in an EnsPire® multimode plate reader (PerkinElmer, Waltham, MA). For measurement of GSSG levels, a 25 μL of the supernatant was incubated at room temperature with 10 μL of N-ethylmaleimide (NEM) (0.04 M) for 30 min at room temperature, and after that, 14 μL of the mixture, were added to 176 μL of NaOH (0.1 N) buffer, following of added 10 μL OPT and incubated for 15 min, using the procedure outlined above for GSH assay. Results were presented as the GSH/GSSG ratio.

2.13 Determination of fruit fly energetic metabolism

Energetic metabolism of Drosophila larvae was determined by colorimetric kits. Larvae were weighted and 0.1 g of larvae was homogenized in 500 μL of 20 mM HEPES buffer, pH 7.0, and centrifuged at 14,000 rpm for 30 min at 4°C. The supernatant was used for analysis of glucose, glycogen and triglycerides levels accordingly to the manufacturer's suggested protocol (Labtest®, MG) with minor modifications. The results were calculated as a percentage in relation to the control group and values were normalized by weight of each larvae group.

2.14 Western blotting

Quantification of of the phosphorylation of mitogen-activated protein kinases (MAPKs), AKT, PARP cleavage and β-actin was performed by Western blotting as described by Ternes et al., (2014) with minor modifications. Groups of larvae (2-3 mg/mL) were mechanically homogenized at 4 °C in 200 μL of buffer (pH 7.0) containing 50 mM Tris, 1 mM EDTA, 20 mM Na_3VO_4, 100 mM sodium fluoride and protease inhibitor cocktail. The homogenate were centrifuged at 3,000 g for 10 min at 4 °C and the supernatants collected. Protein content of larvae was measured and the same volume of 4% SDS stop solution (4% SDS, 50 mM Tris, 100 mM EDTA, pH 6.8) was added to the sample with 25% glycerol sample and 8% β-mercaptoethanol. Proteins were separated using SDS–PAGE 10% gels, and then electrotransferred to nitrocellulose membranes. Membranes were washed in Tris-

buffered saline with Tween (100 mM Tris HCl, 0.9% NaCl and 0.1% Tween-20, pH 7.5) and incubated overnight (4°C) with different primary antibodies, all produced in rabbit (anti-phosphorylated and total ERK1/2, anti-phosphorylated p38, anti-phosphorylated JNK1/2 and anti-β-actin, anti-phosphorylated AKT, and anti-PARP. Following incubation, membranes were washed in Tris-buffered saline with Tween and incubated for 1 h at 25°C with alkaline phosphatase-linked anti rabbit-IgG secondary specific antibodies. The immunoblottings were visualized on the 400MM Pro Bruker Imaging System (Billerica®, MA) using BCIP/NBT Color Development Substrate. Band density was quantified using the Scion Image® Software.

2.15 Caspase assay

Groups of larvae were homogenized in 250 µL of 20 mM HEPES buffer pH 7.0, and centrifuged at 20,000 g for 1 min at 4°C. The supernatant was used for determination of caspases 3/7 activity accordingly to the manufacturer's suggested protocol (Promega®, MA). The results were calculated as a percentage in relation to the control group and values were normalized by protein concentration.

2.16 Quantitative Real-Time qRT-PCR and Gene Expression Analysis

Expression of GPDH, CAT, SOD, TrxR, Nrf2, Drice and Dilps (2,3,5 and 6) genes of Drosophila larvae were analyzed (Table 5). Approximately 1 µg of total RNA from 20 larvae treated with 0.1 mg/mL of HESB was extracted using the Trizol Reagent (Invitrogen®, CA) accordingly to the manufacturer's suggested protocol. After quantification, total RNA was treated with DNase I (DNAseI Amplification Grade - Invitrogen®, NY) and cDNA was synthesized with iScript cDNA Synthesis Kit and random primers again accordingly to the manufacturer's suggested protocol (Biorad®, CA). Quantitative real-time polymerase chain reaction was performed in 11 µL reaction volumes containing water treated with diethyl pyrocarbonate (DEPC), 200 ng of each primer (described in Table 3), and 0.2 x SYBR Green I (molecular probes) using a 7500 real time PCR system (Applied Biosystems®, NY). The qPCR protocol was the following: activation of the reaction at 50°C for 2 min, 95°C for 2 min, followed by 40 cycles of 15 s at 95°C, 60 s at 60°C, and 30 s at 72°C. All samples were analyzed as technical and biological triplicates. Threshold and baselines were automatically determined SYBR fluorescence was analyzed by 7500 software version 2.0.6 (Applied Biosystems®, NY), and the CT (cycle threshold) value for each sample was calculated and reported using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). The GPDH gene was used as

endogenous reference genes presenting no alteration in response to the treatment. For each well, analyzed in triplicates, a ΔCT value was obtained by subtracting the GPDH CT value from the CT value of the interest gene. The ΔCT mean value obtained from the control group of each gene was used to calculate the ΔΔCT of the respective gene ($2^{-\Delta\Delta CT}$).

2.17 Statistical analysis

Lifespan measurement was determined by comparing the survival curves with a log-rank (Mantel–Cox) test. Other statistical analysis was performed using one-way ANOVA and Tukey's *post hoc* test, two-way ANOVA and Bonferroni *post hoc test*, and Unpaired T Test. Differences were considered significant between groups at $p<0.05$ using the GraphPad Prism5 program (La Jolla®, CA). LC_{50} was calculated using PROBIT analysis using Statistics 8 program (Tulsa®, OK).

3. Results

3.1 Qualitative and quantitative analyses of phenolics and flavonoids compounds by HPLC-DAD

The HPLC profile of *S. brasiliensis* extract leaves were acquired, and shown in Fig. 1. The major components in the extract were caffeic acid (3.18 mg/g), gallic acid (2.57 mg/g), and vitexin (1.65 mg/g) (Table 1).

3.2 In vitro antioxidant activity determination

The HESB power antioxidant was assayed by $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical scavenging capacity, total phenolics content and ferric reducing antioxidant power (FRAP) tests. The ABTS neutralizing potential of extract expressed as AAE was (57.32 µM AAE/100 mg) and in DPPH assay (48.69 µM AAE/100 mg). The total phenolic content was 13.61 g of GAE/100 g HESB, and gallic acid was used as a positive control. The ferric reducing antioxidant power (FRAP) of HESB was assayed, the values obtained were 300.4 µM of Fe (II)/100 g of extract. These results are shown in Table 2.

3.3 Effect of HESB on Survival of D. melanogaster

Adult flies were exposed for 7 days with HESB to concentrations of 1, 5, 10, 50, 100 and 200 mg/mL mixed in standard medium. It was not observed significant effect of extract

on fly survival up to 7 days of exposure (Fig. 2). LC_{50} found by Probit analysis was 608.60 mg/mL for adult flies. The individual locomotor activity of flies exposed to HESB was tested, and the treatment did not change the time and tendency of the flies climb to top (Fig. 3). The natural behavior of flies is to fly to the top of a glass tube, which is known as negative geotaxis, and is used to measure the locomotor ability.

3.4 Effect of HESB exposure in the development of Drosophila melanogaster

Drosophila melanogaster eggs, larvae, pupae and hatched flies were exposed to HESB at 0.01, 0.1, 0.5 and 1 mg/mL concentrations mixed in agar medium. We have observed a significant decrease of hatched flies' rate at 1 mg/mL. As observed in Fig. 4, at twentieth day, 28 flies were hatched from 50 eggs at control group, while that for 1 mg/mL, only 1 fly from 50 eggs were ecloded (a decreasing of 96.4 % in eclosion rate). The number of larvae and pupae were not changed in relation to control (data not shown).

3.5 Cellular viability and DCF-DA oxidation assay

The third instar Drosophila larvae treated with HESB 1 mg/mL presented a significant decreasing of 32% and 18% cell viability in MTT and Resazurin assays respectively (Fig. 5. A-B), and a significant induction of 56% in fluorescence levels of DCF-DA oxidation comparing to control (Fig. 6).

3.6 Enzyme assays and determination of glutathione levels

The activity of antioxidant enzymes and GSH/GSSG ratio in Drosophila larvae exposed to HESB 1 mg/mL was significantly altered. We observed a stimulation of 23% in GST and 52% in SOD activities (Fig. 7. A-B), in contrary, a decreasing of 25% in CAT activity (Fig. 7C) was observed followed by an increase of 42% in GSH/GSSG ratio (Fig. 8. A). GSH content was unchanged while that GSSG levels was decreased (Fig. 8. B-C).

3.7 Fruit fly energetic metabolism determination

We determined the effect of exposure to HESB 1 mg/mL in the energetic metabolism of third instar Drosophila larvae, and we can observe a significant decrease of 53% in glucose, 59% in glycogen and of 75% in triglycerides levels (Fig. 9. A-C).

3.8 Phosphorylation of protein kinase and PARP cleavage

The phosphorylation of the protein kinases (ERK1/2, JNK 2, $p38^{MAPK}$, AKT) and PARP cleavage was analysed in the larvae exposed to HESB. ERK2, JNK2, $p38^{MAPK}$ and AKT showed a 31%, 57%, 22% and 66% of inhibition of phosphorylation respectively (Fig. 10. B-E). It was observed a stimulation of 34% in PARP cleavage (Fig. 10. F). β-actin was the loading control for samples (Fig. 10. A).

3.9 Caspase assay

The HESB 1 mg/mL induced caspases 3/7 activity in 79% (Fig. 11).

3.10 Gene Expression Analysis

The treatment with HESB 0.1 mg/mL was able to alter the expression of genes involved to cellular homeostasis, oxidative stress, apoptosis, and metabolism regulation in *D. melanogaster* larvae. qRT-PCR analysis revealed a significant increase in gene expression of CAT 1.57 fold, TrxR 1.57 fold, Nrf2 1.56 fold, Drice 3.84 fold and Dilp6 1.34 fold (Table 4). The SOD and Dilps (2,3,5) genes no demonstrated differences in the expression compared to control (Table 4).

4. Discussion

The present study was performed to investigate the potencial toxicity of crude extract of leaves of *Senecio brasiliensis* in *Drosophila melanogaster* in different developmental stages and biochemical and molecular targets of the extract. Since major mechanisms of detoxification of *Drosophila melanogaster* are similar to humans, Drosophila can be an essential model to understanding role of important molecules for homeostasis and toxicity response (Jennings, 2011; Rand et al., 2014). HESB treatment did not induce mortality or locomotor alterations in adulthood of *D. melanogaster*. Nevertheless, we demonstrated for the first time the ability of *Senecio brasiliensis* to influence the development and considerably impair the eclosion process in *Drosophila melanogaster*. We observed a significant decrease in eclosion flies rate in Drosophila exposed to 1 mg/mL of HESB, without changing the number of larvae and pupae in relation to control. Thus, the process of hatching was disrupted, although the developmental processes of larvae occured normally in the presence of extract.

The initiation of hatching is deflagrated by a complex of hormones and signaling pro-

teins. With the end of larval third instar starts the metamorphosis process, that culminate with the activation of four hormones that are implicated in the ecdysis and eclosion process: ETH (ecdysis triggering hormone), that activate the EH (eclosion hormone), CCAP (crustacean cardioactive peptide) and Bursicon on CNS (Kruger et al., 2015). Thus, a modulation in this array by potentially toxic compounds could impair this process decreasing the number of adult flies. Thus in this study it was investigated genetical and biochemical alterations by extract in oxidative stress and kinases with recognized involvement in developmental process.

Several indicators of a condition of oxidative stress were present in larvae grown in HESB supplemented medium. Oxidative stress is a condition caused by the excessive production of free radicals in counterpoint with insufficient antioxidant defense systems of organism. This condition causes numerous alterations in biological molecules, mainly DNA and protein damages (Birben et al., 2012). Enzymatic and nonenzymatic cellular defense and activation in transcriptional factors are implied in cell response to ROS. In this study was evidenced the overproduction of ROS in relation to control in larval phase. An increased ratio GSH/GSSG, GST and SOD activity additionally with induction of Nrf2, CAT and TrxR gene expression could be a response to ROS induction that was potentiated by decrease in catalase activity. CAT, SOD and GST are part of the antioxidant enzymatic system (Kabil et al., 2007; Low et al., 2007; Park et al., 2004). GST assists in the excretion of xenobiotics, plant allelochemicals, and ROS of the organism, converting them to less toxic and water soluble forms, through the conjugation of these toxics with GSH (Low et al., 2007). Glutathione participates of the non enzymatic antioxidant capacity, and in its reduced state (GSH) is one of the most important scavengers of ROS, acting at detoxification of the organism (Aquilano et al., 2014). The catalytic cycle of glutathione is critical for maintaining cell homeostasis, and GSH/GSSG ratio may be used as an indicator of cell redox state (Owen and Butterfield, 2010). The significant increase of GSH/GSSG ratio, SOD and GST activities observed in larvae treated with HESB could represent an adaptive cellular response against HESB toxicity, acting in the detoxification of the H_2O_2 that CAT was not capable to act. The decline in CAT activity is related with a state of antioxidant dysfunction (Sullivan-Gunn and Lewandowski, 2013). A study revealed that lack catalase activity in *D. melanogaster* is associated with a diminution of life span after eclosion (DeJong et al., 2007), thus the decreasing in CAT activity showed here could be in part involved in the augmented oxidative stress and lead to shortened life span or even avoid the eclosion. Moreover, its inhibition by extract seems to be a post transductional event, since the gene expression of this enzyme was

augmented.

The Nrf2-ARE signaling pathway is a mechanism that regulates the expression of a variety of defense enzymes (Pitoniak and Bohmann, 2015). In the nucleus, Nrf2 binds to the ARE (antioxidant response element), activating the transcription of antioxidant enzymes phase II detoxification (thioredoxins, glutathione S-transferases, glutathione-synthesizing enzymes and others) which maintain the redox balance (Gorrini et al., 2013; Pitoniak and Bohmann, 2015; Zhang et al., 2013). Besides, in basal conditions, Nrf2 activity also maintains housekeeping expression of the same antioxidant and detoxification genes (Sykiotis and Bohmann, 2009). In this study, Nrf2, TrxR gene expression and GST activity were increased suggesting the involvement of Nrf2pathway in the antioxidant response of cell to the insult. In *D. melanogaster*, the thioredoxin system is responsible for GSSG reduction (Kanzok et al., 2001). TrxR has a fundamental role acting against ROS and interacting with SOD and CAT (Missirlis et al., 2001). Based in our results it may suppose that augmented TrxR gene expression could improve the TrxR protein synthesis thus diminishing the GSSG level as seen in this study.

According to the experiments, the larvae feeding with 1 mg/mL of HESB had decreased metabolic viability. In agreement with this found, it was demonstrated stimulation of effectors caspases 3 and 7 activity and cleavage of PARP proteins suggesting induction of apoptotic cell death in larvae. In parallel, Drice gene expression, a fly homologous of the mammal caspases 3 and 7 was inducted. Apoptosis is a highly regulated programmed cell death process dependent on specific cellular signaling (McIlwain et al., 2013). Caspases proteins (cysteine-dependent aspartate-specific proteases) have central role in this apoptotic mechanism, and are activated in stress conditions (Van De Water et al., 2004). Drosophila and mammals have many apoptotic conserved aspects. Mammals possess three effector caspases (3, 6 and 7) (McIlwain et al., 2013), while *D. melanogaster* has four effectors (Dcp-1, Drice, Decay, and Damm) (Hay and Guo, 2006). Caspase 3 is able to cleave and inactivate Poly (ADP-ribose) polymerase (PARP) protein that plays important functions on detection and repair of DNA damage and helps to maintain genome integrity (Morales et al., 2014).

The MAPK (Mitogen-activated protein kinases) pathways are evolutionarily conserved in eukaryotic cells and mediate essentials cellular process and their activity is regulated by phosphorylation (Kim and Choi, 2010). ERK (extracellular signal-regulated kinase) is related to survival and cell proliferation, while JNK (cJun N-terminal kinase) can

perform both proliferation and apoptosis, and $p38^{MAPK}$ is able to block proliferation and promove to apoptosis (Kennedy et al., 2007). Oxidative stress can activate or inhibit the MAPK pathways (Son et al., 2009, 2011). The $p38^{MAPK}$ and JNK activation is regulated by MAPK kinase kinase ASK1. In basal conditions ASK1 remains associate with Thioredoxin (Trx) in reduced form, however in stress conditions $p38^{MAPK}$ and JNK are activated after oxidation and dissossiation of Trx. ROS also can inhibite the $p38^{MAPK}$ and JNK pathways, through the modulation of the phosphatases that inactive ASK1, and consequently inactive $p38^{MAPK}$ and JNK (Hayat, 2013) (Kennedy et al., 2007). It has been demonstrated that ERK pathway is able to stimulate the expression of important pro survival proteins such as Bcl2 and Bclx (Dang et al., 2015). ERK pathway can be both activated and inhibited by ROS (Cagnol and Chambard, 2010). MAPK cascades are implied in important aspects of *D. melanogaster* development over embryos to larval stages such as the development of eye and wing, cytoskeletal changes, wing morphogenesis and insect immunity (Shilo, 2014). Augmented phosphorylation of these kinases by heavy metals and exposure to hydroalcoholic extract of plants was previously demonstrated in Drosophila (Paula et al., 2012; Pinho et al., 2014). AKT has an important role in cellular physiology, survival, growth, stress response and glucose metabolism (Manning and Cantley, 2007). In *D. melanogaster*, AKT acts as a survival protein, involved in cell growth, aging and stress responses. Growth factors and insulin are able to activate AKT by PI3K (phosphatidylinositol-3-OH kinase) signaling pathway (Scanga et al., 2000). Our results show that *Senecio brasiliensis* exposure inhibited the MAPK and AKT signaling pathway in *Drosophila melanogaster* larvae which may by disrupting the normal development of the fruit fly and its eclosion from the pupa.

The insulin signaling in *D. melanogaster* is required for the regulation of cell growth, being the energetic metabolism essential for correct development of fruit fly (Haselton and Fridell, 2010). Drosophila has insulin-like peptides known as Dilps (Drosophila insulin-like peptides) that possess structural and functionally similarities with Insulin Growth Factor 1 (IGF-1), insulin, and insulin/IGF1 signaling (IIS) pathway (Kannan and Fridell, 2013), and act in important functions, as regulation of developmental growth, stress resistance, regulation of glucose metabolic process and in insulin receptor signaling pathway (Kannan and Fridell, 2013). It was observed a significant increase on Dilp6 gene expression. Dilp6 is involved in biological processes as positive regulation of growth during development (Bai et al., 2012). Has been reported that gene Dilp6 is expressed in fat body cells, and has its increased expression during larval and pupal stages and in starvation conditions (Slaidina et al., 2009).

Perhaps the extract diluted at culture medium had proportioned a malnutrition condition and occasioning the increasing of Dilp6 gene expression as an adaptive response to feeding pressure. Accordingly, we have analyzed the energetic metabolism of the treated larvae. Glycogen serves as a major reserve source of glucose in *D. melanogaster*, and plays a key role in muscle activity related to flight, mating, oviposition, and assists in survival in hostile environments (Foley and Luckinbill, 2001). In holometabolous insects, as *D. melanogaster*, the maximum glycogen content is reached at the end of the larval period and is used as a source of glucose and energy, decreasing along the metamorphosis. In the pupal stage a large quantity of nutrients stored is necessary to prolong growth and finalize the development of adult structures (Arrese and Soulages, 2010). Our data shows a diminution of glucose, glycogen and triglycerides levels in larvae, exposed to HESB, fact that could be associated with lower rate of flies hatching.

Studies have reported that presence of *Senecio brasiliensis* extracts decreases oviposition of *Bemisia tabaci* (Ribeiro et al., 2009) and causes larvicity in *Aedes fluviatilis* (Macêdo et al., 1997). Another species of the genus also have presented toxic effects, as insect antifeedant activity of *Senecio miser* (Reina et al., 2001); insecticidal activity of *Senecio salignus* in *Spodoptera frugiperda* (Romo-asunción et al., 2016) and larvicidal activity of *Senecio pterophorus* in *Culex quinquefascitus* (Lawal et al., 2016). In accordance, our data shows the toxic effects to *S. brasiliensis* specifically on eclosion rate of fly model *D. melanogaster*. The vegetal secondary metabolism is responsible to synthesize molecules with different biological characteristics, as therapeutic and defensives, extremely useful in biological research (Maag et al., 2015; War et al., 2012). The HPLC analysis revealed the presence of different flavonoids such as vitexin, caffeic and gallic acids. Total amount of phenolics were also detected in HESB. Studies have demonstrated that polyphenols such as phenolic acids and flavonoids can modulate cellular functions as cell signaling and maintaining of redox state of cells as well as to lead to inhibition of enzymatic systems involved in the detoxification of xenobiotics and ROS (Kuete, 2014) (Sotibrán et al., 2011). Previous studies showed that a mixture of quercetin and gallic acid has strong antifungical potential, and quercetin with ferulic acid demonstrated insecticidal activity in *Drosophila melanogaster* (Céspedes et al., 2014). Additionally cytotoxic, mutagenic, and carcinogenic effects was attributed to quercetin (Sotibrán et al., 2011) and antiviral and antifungical effects to caffeic acid (Arakawa et al., 2009; Pukkila-Worley et al., 2009). Thus, the toxicity of HESB could be attributed to a synergistic interaction conferred by the presence of different

polyphenols in the extract such as polyphenols and alkaloids, this last although not identified, its presence is well described in literature for this gender (Tundis et al., 2007).

The *in vitro* antioxidant potential of HESB facing DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals was checked. The average values for ABTS assay (57.3±4.01 μM of AAE/100 mg) was higher than those observed for DPPH (48.7±7.25 μM of AAE/100 mg), which was expected since ABTS assay reacts with both lipophilic and hydrophilic antioxidants (Re et al., 1999). The HESB presented iron reducing power (300.4±11.36 μM of FeII/100 g). The values obtained for both radicals was lower than for other species studied by our research group, such as *Psidium guajava* (74.76±18.06 and 78.04±5.76 μM of AAE/100 mg of extract, by ABTS and DPPH assays respectively) (Rodrigues et al., 2015).

5. Conclusions

Our study demonstrated the toxicity of the leaves of *S. brasiliensis* and its capacity to affect the eclosion rate of adult *D. melanogaster*. Additionally it was investigated biochemical biomarkers potentially involved in stress response and development of Drosophila. A pletora of targets are modulated by constituents present in *S. brasiliensis* (Scheme). This modulation could be related to oxidative stress resulted from toxic constituents of extract or a direct action of this constituent on cellular molecules. The potentiality of this plant to causes apoptosis and to inhibit survival pathway points out to a possible antiproliferative effect of *S. brasiliensis.*

Conflict of interest

The authors declare that there is no conflict of interest.

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References

Aebi, H., 1984. Oxygen Radicals in Biological Systems. Methods Enzymol. 105, 121–126.

Aquilano, K., Baldelli, S., Ciriolo, M.R., 2014. Glutathione: New roles in redox signalling for an old antioxidant. Front. Pharmacol. 5 AUG, 1–12.

Arakawa, T., Yamasaki, H., Ikeda, K., Ejima, D., Naito, T., Hajime Koyama, A., 2009. Antiviral and Virucidal Activities of Natural Products. Curr. Med. Chem. 16, 2485–2497.

Arrese, E.L., Soulages, J.L., 2010. Insect Fat Body: Energy, Metabolism, and Regulation. Annu. Rev. Entomol. 55, 207–225.

Bai, H., Kang, P., Tatar, M., 2012. Drosophila insulin-like peptide-6 (dilp6) expression from fat body extends lifespan and represses secretion of Drosophila insulin-like peptide-2 from the brain. Aging Cell 11, 978–985.

Baltrušaityte, V., Venskutonis, P.R., Čeksteryte, V., 2007. Radical scavenging activity of different floral origin honey and beebread phenolic extracts. Food Chem. 101, 502–514.

Benzie, I.., Strain, J.., 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal. Biochem. 239, 70–6.

Birben, E., Murat, U., Md, S., Sackesen, C., Erzurum, S., Kalayci, O., 2012. Oxidative Stress and Antioxidant Defense. WAO J. 5, 9–19.

Birse, R.T., Söderberg, J.A.E., Luo, J., Winther, A.M.E., Nässel, D.R., 2011. Regulation of insulin-producing cells in the adult Drosophila brain via the tachykinin peptide receptor DTKR. J. Exp. Biol. 214, 4201–8.

Boligon, A.A., Piana, M., Kubiça, T.F., Mario, D.N., Dalmolin, T.V., Bonez, P.C., Weiblen, R., Lovato, L., Alves, S.H., Campos, M.M.A., Athayde, M.L., 2015. HPLC analysis and antimicrobial, antimycobacterial and antiviral activities of *Tabernaemontana catharinensis* A. DC. J. Appl. Biomed. 13, 7–18.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Brito, S.M.O., Coutinho, H.D.M., Talvani, A., Coronel, C., Barbosa, A.G.R., Vega, C., Figueredo, F.G., Tintino, S.R., Lima, L.F., Boligon, A.A., Athayde, M.L., Menezes, I.R.A., 2015. Analysis of bioactivities and chemical composition of *Ziziphus joazeiro* Mart. using HPLC-DAD. Food Chem. 186, 185–191.

Bryan, H.K., Olayanju, A., Goldring, C.E., Park, B.K., 2013. The Nrf2 cell defence pathway: Keap1-dependent and -independent mechanisms of regulation. Biochem. Pharmacol. 85, 705–717.

Cagnol, S., Chambard, J.C., 2010. ERK and cell death: Mechanisms of ERK-induced cell death - Apoptosis, autophagy and senescence. FEBS J. 277, 2–21.

Cespedes, C.L., Aqueveque, P.M., Avila, J.G., Alarcon, J., Kubo, I., 2015. New advances in chemical defenses of plants: researches in calceolariaceae. Phytochem. Rev. 14, 367–

380.

Céspedes, C.L., Salazar, J.R., Ariza-Castolo, A., Yamaguchi, L., Ávila, J.G., Aqueveque, P., Kubo, I., Alarcón, J., 2014. Biopesticides from plants: *Calceolaria integrifolia* s.l. Environ. Res. 132, 391–406.

Chatterjee, D., Katewa, S.D., Qi, Y., Jackson, S.A., Kapahi, P., Jasper, H., 2014. Control of metabolic adaptation to fasting by dILP6-induced insulin signaling in Drosophila oenocytes. Proc. Natl. Acad. Sci. U. S. A. 111, 17959–64.

Chen, B., Lu, Y., Chen, Y., Cheng, J., 2015. The role of Nrf2 in oxidative stress-induced endothelial injuries. J. Endocrinol. 225, R83–R99.

Cruz, L.C., Batista, J.E.S., Zemolin, A.P.P., Nunes, M.E.M., Lippert, D.B., Royes, L.F.F., Soares, F.A., Pereira, A.B., Posser, T., Franco, J.L., 2014. A study on the quality and identity of Brazilian Pampa biome honey: Evidences for its beneficial effects against oxidative stress and hyperglycemia. Int. J. Food Sci. 2014.

Dang, S., Yu, Z.-M., Zhang, C.-Y., Zheng, J., Li, K.-L., Wu, Y., Qian, L.-L., Yang, Z.-Y., Li, X.-R., Zhang, Y., Wang, R.-X., 2015. Autophagy promotes apoptosis of mesenchymal stem cells under inflammatory microenvironment. Stem Cell Res. Ther. 6, 247.

De Souza, R.R., Bretanha, L.C., Dalmarco, E.M., Pizzolatti, M.G., Fröde, T.S., 2015. Modulatory effect of *Senecio brasiliensis* (Spreng) Less. in a murine model of inflammation induced by carrageenan into the pleural cavity. J. Ethnopharmacol. 168, 373–379.

DeJong, R.J., Miller, L.M., Molina-Cruz, A., Gupta, L., Kumar, S., Barillas-Mury, C., 2007. Reactive oxygen species detoxification by catalase is a major determinant of fecundity in the mosquito *Anopheles gambiae*. Proc. Natl. Acad. Sci. U. S. A. 104, 2121–2126.

Foley, P. a, Luckinbill, L.S., 2001. The effects of selection for larval behavior on adult life-history features in *Drosophila melanogaster*. Evolution 55, 2493–2502.

Gorrini, C., Harris, I.S., Mak, T.W., 2013. Modulation of oxidative stress as an anticancer strategy. Nat. Rev. Drug Discov. 12, 931–47.

Grönke, S., Clarke, D.-F., Broughton, S., Andrews, T.D., Partridge, L., 2010. Molecular evolution and functional characterization of Drosophila insulin-like peptides. PLoS Genet. 6, e1000857.

Habig, W.H., Jakoby, W.B., 1981. Glutathione S-transferases (rat and human). Methods Enzymol. 77, 218–231.

Haselton, A.T., Fridell, Y.-W.C., 2010. Adult *Drosophila melanogaster* as a model for the study of glucose homeostasis. Aging (Albany. NY). 2, 523–6.

Hay, B.A., Guo, M., 2006. Caspase-Dependent Cell Death in Drosophila. Annu. Rev. Cell Dev. Biol. 22, 623–650.

Hayat, M.A., 2013. Autophagy: cancer, other pathologies, inflammation, immunity, infection, and aging: v. 1. Molecular mechanisms. 1st ed. Academic Press, Union, NJ.

Hissin, P.J., Hilf, R., 1976. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal. Biochem. 74, 214–226.

Jennings, B.H., 2011. Drosophila a versatile model in biology & medicine. Mater. Today 14, 190–195.

Jimenez-Del-Rio, M., Guzman-Martinez, C., Velez-Pardo, C., 2010. The effects of polyphenols on survival and locomotor activity in *Drosophila melanogaster* exposed to iron and paraquat. Neurochem. Res. 35, 227–238.

Kabil, H., Partridge, L., Harshman, L.G., 2007. Superoxide dismutase activities in long-lived *Drosophila melanogaster* females: Chico1 genotypes and dietary dilution. Biogerontology 8, 201–208.

Kannan, K., Fridell, Y.-W.C., 2013. Functional implications of Drosophila insulin-like peptides in metabolism, aging, and dietary restriction. Front. Physiol. 4, 288.

Kanzok, S.M., Fechner, A., Bauer, H., Ulschmid, J.K., Müller, H.-M., Munoz-Botella, J., Schneuwly, S., Schirmer, R.H., Becker, K., 2001. Substitution of the thioredoxin svstem for glutathione reductase in *Drosophila melanogaster*. Science (80-.). 291, 643–646.

Karam, F.C., Schild, A.L., de Mello, J.R.B., 2011. Intoxicação por Senecio spp. em bovinos no Rio Grande do Sul: Condições ambientais favoráveis e medidas de controle. Pesqui. Vet. Bras. 31, 603–609.

Kennedy, N.J., Cellurale, C., Davis, R.J., 2007. A Radical Role for p38 MAPK in Tumor Initiation. Cancer Cell 11, 101–103.

Kim, E.K., Choi, E.-J., 2010. Pathological roles of MAPK signaling pathways in human diseases. Biochim. Biophys. Acta 1802, 396–405.

Kostyuk, V.A., Potapovich, A.I., 1989. Superoxide--driven oxidation of quercetin and a simple sensitive assay for determination of superoxide dismutase. Biochem. Int. 19, 1117–24.

Krüger, E., Mena, W., Lahr, E.C., Johnson, E.C., Ewer, J., 2015. Genetic analysis of Eclosion hormone action during Drosophila larval ecdysis. Development 4279–4287.

Kuete, V., 2014. Toxicological survey of African medicinal plants. 1st ed.Elsevier Inc, Dschang, ME.

Lawal, O., Ogunwande, I., Mzimela, H., Opoku, A., Oyedeji, A., 2016. *Senecio pterophorus*

DC. (Asteraceae) Essential Oils: Antibacterial, Antioxidant, Cytotoxic and Larvicidal Activities. Br. J. Pharm. Res. 12, 1–11.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and. Methods 25, 402–408.

Lizarraga, E.. b, Castro, F.., Fernández, F.., De Lampasona, M.P.., Catalán, C.A.N.., 2012. Antioxidant, hemolytic and cytotoxic activities of Senecio species used in traditional medicine of northwestern Argentina. Nat. Prod. Commun. 7, 607–608.

Lone, S.H., Bhat, K.A., Bhat, H.M., Majeed, R., Anand, R., Hamid, A., Khuroo, M.A., 2014. Essential oil composition of *Senecio graciliflorus* DC: Comparative analysis of different parts and evaluation of antioxidant and cytotoxic activities. Phytomedicine 21, 919–925.

Low, Y.W., Ng, H.L., Morton, C.J., Parker, M.W., Batterham, P., Robin, C., 2007. Molecular evolution of glutathione S-transferases in the genus Drosophila. Genetics 177, 1363–1375.

Maag, D., Erb, M., Köllner, T.G., Gershenzon, J., 2015. Defensive weapons and defense signals in plants: Some metabolites serve both roles. BioEssays 37, 167–174.

Macêdo, M.E., Consoli, R. a G.B., Grandi, T.S.M., Dos Anjos, A.M.G., De Oliveira, A.B., Mendes, N.M., Queiróz, R.O., Zani, C.L., 1997. Screening of Asteraceae (Compositae) Plant Extracts for Larvicidal Activity against *Aedes fluviatilis* (Diptera: Culicidae). Mem. Inst. Oswaldo Cruz 92, 565–570.

Manning, B., Cantley, L., 2007. AKT/PKB Signalling: Navigating Downstream. Cell 129, 1261–1274.

Manubolu, M., Goodla, L., Ravilla, S., Obulum, V.R., 2013. Activity-guided isolation and identification of anti-staphylococcal components from *Senecio tenuifolius* Burm. F. leaf extracts. Asian Pac. J. Trop. Biomed. 3, 191–195.

Matos, F.J.A., 1997. Introdução a fitoquímica experimental. 3rd ed. UFC Editions., Fortaleza, CE.

McIlwain, D.R., Berger, T., Mak, T.W., 2013. Caspase functions in cell death and disease. Cold Spring Harb. Perspect. Biol. 5, 1–28.

Mierziak, J., Kostyn, K., Kulma, A., 2014. Flavonoids as important molecules of plant interactions with the environment. Molecules 19, 16240–16265.

Mishra, B.B., Tiwari, V.K., 2011. Natural products: An evolving role in future drug discovery. Eur. J. Med. Chem. 46, 4769–4807.

Missirlis, F., Phillips, J.P., Jäckle, H., 2001. Cooperative action of antioxidant defense systems in Drosophila. Curr. Biol. 11, 1272–1277.

Mollinedo, P., Vila, J.L., Arando, H., Sauvain, M., Deharo, E., Bravo, J.A., Mollinedo, P., Arando, H., Sauvain, M., Deharo, E., Sauvain, M., Deharo, E., 2015. Anti-infective assessment of *Senecio smithioides* (Asteraceae) and isolation of 9-oxoeuryopsin, a furanoeremophilane-type sesquiterpene with antiplasmodial activity. Nat. Prod. Res. 6419, 1–4.

Morales, J.C., Li, L., Fattah, F.J., Dong, Y., Bey, E.A., Patel, M., Gao, J., Boothman, D.A., 2014. Review of Poly (ADP-ribose) Polymerase (PARP) Mechanisms of Action and Rationale for Targeting in Cancer and Other Diseases. Crit Rev Eukaryot Gene Expr 24, 15–28.

O'Brien, J., Wilson, I., Orton, T., Pognan, F., 2000. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur. J. Biochem. 267, 5421–5426.

Owen, J.B.; Butterfield, D.A., 2010. Measurement of oxidized/reduced glutathione ratio. Methods in Molecular Biology. 648, 169-277.

Park, S.Y., Kim, Y.S., Yang, D.J., Yoo, M.A., 2004. Transcriptional regulation of the Drosophila catalase gene by the DRE/DREF system. Nucleic Acids Res. 32, 1318–1324.

Paula, M.T., Zemolin, A.P., Vargas, A.P., Golombieski, R.M., Loreto, E.L.S., P, S.A., S, P.R., M, F.E.M., B, P.A., Rocha, J.B.T., Merrit, T.J.S., Franco, J.L., Posser, T., 2012. Effects of Hg(II) Exposure on MAPK Phosphorylation and Antioxidant System in *D. melanogaster.* Environ. Toxicol. 24, 296–303.

Pérez-Severiano, F., Rodríguez-Pérez, M., Pedraza-Chaverrí, J., Maldonado, P.D., Medina-Campos, O.N., Ortíz-Plata, A., Sánchez-García, A., Villeda-Hernández, J., Galván-Arzate, S., Aguilera, P., Santamaría, A., 2004. S-Allylcysteine, a garlic-derived antioxidant, ameliorates quinolinic acid-induced neurotoxicity and oxidative damage in rats. Neurochem. Int. 45, 1175–1183.

Pérez González, C., Serrano Vega, R., González-Chávez, M., Zavala Sánchez, M.A., Pérez Gutiérrez, S., 2013. Anti-inflammatory activity and composition of *Senecio salignus* kunth. Biomed Res. Int. 2013.

Peterson, G.L., 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem. 83, 346-56.

Piljac-Žegarac, J., Stipčević, T., Belščak, A., 2009. Antioxidant properties and phenolic content of different floral origin honeys. J. ApiProduct ApiMedical Sci. 1, 43–50.

Pinho, F.V.S.D.A., Felipe Da Silva, G., Macedo, G.E., Muller, K.R., Martins, I.K., Ternes, A.P.L., Da Costa, J.G.M., Athayde, M.L., Boligon, A.A., Kamdem, J.P., Franco, J.L., De

Menezes, I.R.A., Posser, T., 2014. Phytochemical constituents and toxicity of *Duguetia furfuracea* hydroalcoholic extract in *Drosophila melanogaster.* Evidence-based Complement. Altern. Med. 2014.

Pisoschi, A.M., Pop, A., 2015. The role of antioxidants in the chemistry of oxidative stress: A review. Eur. J. Med. Chem. 97, 55–74.

Pitoniak, A., Bohmann, D., 2015. Mechanisms and functions of Nrf2 signaling in Drosophila. Free Radic. Biol. Med. 88, 302–313.

Pukkila-Worley, R., Holson, E., Wagner, F., Mylonakis, E., 2009. Antifungal drug discovery through the study of invertebrate model hosts. Curr. Med. Chem. 16, 1588–95.

Rand, M.D., Montgomery, S.L., Prince, L., Vorojeikina, D., 2014. Developmental toxicity assays using the Drosophila model. Curr. Protoc. Toxicol. 59, 1.12.1-20.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant Activity Applying an Improved Abts Radical Cation Decolorization Assay. Free Radic. Biol. Med. 26, 1231–1237.

Reina, M., González-Coloma, A., Gutiérrez, C., Cabrera, R., Rodríguez, M.L., Fajardo, V., Villarroel, L., 2001. Defensive chemistry of *Senecio miser*. J. Nat. Prod. 64, 6–11.

Ribeiro, L. do P., Vasconcelos, C.J., Vendramin, J.D., Oriani, M. de G., Lissner, R., 2009. Effect of botanical insecticides on the attractiveness and oviposition preference of *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) in tomato. Rev. Bras. Agroecol. 4, 458–461.

Rodrigues, N.R., Batista, J.E. dos S., de Souza, L.R., Martins, I.K., Macedo, G.E., da Cruz, L.C., da Costa Silva, D.G., Pinho, A.I., Coutinho, H.D.M., Wallau, G.L., Posser, T., Franco, J.L., 2015. Activation of p38MAPK and NRF2 signaling pathways in the toxicity induced by chlorpyrifos in *Drosophila melanogaster*: Protective effects of *Psidium guajava* pomífera L. (Myrtaceae) hydroalcoholic extract. Arab. J. Chem.

Romo-asunción, D., Ávila-calderón, M.A., Ramos-lópez, M.A., Barranco-florido, E., Rodríguez-Navarro, S., Romero-Gomez, S., Aldeco-Pérez, E.J., Pacheco-Aguilar, J.R., Rico-Rodríguez, M.A., 2016. Juvenomimetic and Insecticidal Activities of *Senecio salignus* (Asteraceae) and *Salvia microphylla* (Lamiaceae) on *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Florida Entomol. Soc. 99, 345–351.

Sandini, T.M., Udo, M.S.B., Reis-Silva, T.M., Sanches, D., Bernardi, M.M., Flório, J.C., Spinosa, H. de S., 2015. Prenatal exposure to integerrimine N-oxide enriched butanolic residue from *Senecio brasiliensis* affects behavior and striatal neurotransmitter levels of rats in adulthood. Int. J. Dev. Neurosci. 47, 157–164.

Sen, T., Samanta, S.K., 2015. Medicinal Plants, Human Health and Biodiversity: A Broad Review. Adv Biochem Eng Biotechnol.147, 59-110.

Scanga, S.E., Ruel, L., Binari, R.C., Snow, B., Stambolic, V., Bouchard, D., Peters, M., Calvieri, B., Mak, T.W., Woodgett, J.R., Manoukian, A.S., 2000. The conserved PI3'K/PTEN/Akt signaling pathway regulates both cell size and survival in Drosophila. Oncogene 19, 3971–7.

Shilo, B.Z., 2014. The regulation and functions of MAPK pathways in Drosophila. Methods 68, 151–159.

Slaidina, M., Delanoue, R., Gronke, S., Partridge, L., Léopold, P., 2009. A Drosophila Insulin-like Peptide Promotes Growth during Nonfeeding States. Dev. Cell 17, 874–884.

Son, T.G., Camandola, S., Mattson, M.P., 2009. Hormetic Dietary Phytochemicals. Neuromolecular Med 10, 236–246.

Son, Y., Cheong, Y.-K., Kim, N.-H., Chung, H.-T., Kang, D.G., Pae, H.-O., 2011. Mitogen-Activated Protein Kinases and Reactive Oxygen Species: How Can ROS Activate MAPK Pathways? J. Signal Transduct. 2011, 792639.

Sotibrán, A.N.C., Ordaz-Téllez, M.G., Rodríguez-Arnaiz, R., 2011. Flavonoids and oxidative stress in *Drosophila melanogaster.* Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 726, 60–65.

Sullivan-Gunn, M.J., Lewandowski, P. a, 2013. Elevated hydrogen peroxide and decreased catalase and glutathione peroxidase protection are associated with aging sarcopenia. BMC Geriatr. 13, 104.

Sykiotis, G.P., Bohmann, D., 2009. Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in Drosophila. Dev. Cell 14, 76–85.

Ternes, A.P.L., Zemolin, A.P., Cruz, L.C., Silva, G.F., Saidelles, A.P.F., Paula, M.T. De, Wagner, C., Golombieski, R.M., Flores, É.M. de M., Picoloto, R.S., Pereira, A.B., Franco, J.L., Posser, T., 2014. Original article : *Drosophila melanogaster* – an embryonic model for studying behavioral and biochemical effects of manganese exposure. EXCLI J. 13, 1239–1253.

Tidjani, S., Okusa, P.N., Zellagui, A., Banuls, L.M.Y., Stévigny, C., Duez, P., Rhouati, S., 2013. Analysis of pyrrolizidine alkaloids and evaluation of some biological activities of Algerian *Senecio delphinifolius* (Asteraceae). Nat. Prod. Commun. 8, 439–40.

Tiwari, R., Rana, C.S., 2015. Plant secondary metabolites: a review. Int. J. Eng. Res. Gen. Sci. 3, 661–670.

Toma, W., Trigo, J.R., de Paula, A.C.B., Brito, A., 2004. Modulation of gastrin and epidermal

growth factor by pyrrolizidine alkaloids obtained from *Senecio brasiliensis* in acute and chronic induced gastric ulcers. Can. J. Physiol. Pharmacol. 82, 319–325.

Tundis, R., Loizzo, M.R., Statti, G.A., Passalacqua, N.G., Peruzzi, L., Menichini, F., 2007. Pyrrolizidine alkaloid profiles of the *Senecio cineraria* Group (Asteraceae). Zeitschrift fur Naturforsch. - Sect. C J. Biosci. 62, 467–472.

Van De Water, T.R., Lallemend, F., Eshraghi, a a, Ahsan, S., He, J., Guzman, J., Polak, M., Malgrange, B., Lefebvre, P.P., Staecker, H., Balkany, T.J., 2004. Caspases, the enemy within, and their role in oxidative stress-induced apoptosis of inner ear sensory cells. Otol. Neurotol. 25, 627–32.

War, A.R., Paulraj, M.G., Ahmad, T., Buhroo, A.A., Hussain, B., Ignacimuthu, S., Sharma, H.C., 2012. Mechanisms of plant defense against insect herbivores. Plant Signal. Behav. 7, 1306–20.

Xiao, K.-J., Wang, W.-X., Dai, J.-L., Zhu, L., 2014. Anti-inflammatory activity and chemical composition of the essential oils from *Senecio flammeus*. EXCLI J. 13, 782–791.

Zhang, M., An, C., Gao, Y., Leak, R.K., Chen, J., Zhang, F., 2013. Emerging roles of Nrf2 and phase II antioxidant enzymes in neuroprotection. Prog. Neurobiol. 100, 30–47.

Table 1: Phenolics and flavonoids components of leaves of *S. brasiliensis* extract

Coumpounds	**HESB (mg/g)**	**%**	**LOD (µg/mL)**	**LOQ (µg/mL)**
Gallic acid	2.57 ± 0.01 a	0.25	0.023	0.076
Chlorogenic acid	1.63 ± 0.01 b	0.16	0.011	0.036
Caffeic acid	3.18 ± 0.03 c	0.31	0.028	0.094
Rosmarinic acid	1.40 ± 0.02 d	0.14	0.019	0.063
Vitexin	1.65 ± 0.04 b	0.16	0.015	0.049
Quercetin	0.42 ± 0.01 e	0.04	0.024	0.080

Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.05$.

Table 2: Antioxidant activity of Hydroalcoholic extract of leaves of *S. brasiliensis in vitro*

DPPH (µM AAE/100 mg)	**ABTS (µM AAE/100 mg)**	**Phenols (g of GAE/100 g)**	**FRAP (µM of FE(II)/100g)**
48.69±7.25	57.32±4.01	13.61±4.1	300.4±11.36

Data are expressed as mean ± SD of three determinations.

Table 3: Genes tested by quantitative real-time RT-PCR analysis and forward and reverse primers

Gene		**Primer Sequences**
GPDH	LEFT	5’ – ATGGAGATGATTCGCTTCGT
	RIGHT	5’ - GCTCCTCAATGGTTTTTCCA
SOD	LEFT	5’ – GGAGTCGGTGATGTTGACCT
	RIGHT	5’ - GTTCGGTGACAACACCAATG
CAT	LEFT	5’- ACCAGGGCATCAAGAATCTG
	RIGHT	5’ - AACTTCTTGGCCTGCTCGTA
TrxR-1	LEFT	5’ – CGTTCTATTGTGCTGCGTGG
	RIGHT	5’ - AGCTTGCCATCATCCTGCTT
Nrf2	LEFT	5' – CGTGTTGTTACCCTCGGACT
	RIGHT	5' - AGCGCATCTCGAACAAGTTT
Drice	LEFT	5' – GTCGCAGAATCACAGCGATA
	RIGHT	5' - GGCAGGCCTGTATGAAGAAC
Dilp 2	LEFT	5’ – ATCCCGTGATTCCACACAAG
	RIGHT	5’ - GCGGTTCCGATATCGAGTTA
Dilp 3	LEFT	5’ – CCGAAACTCTCTCCAAGCTC
	RIGHT	5’ - GCCATCGATCTGATTGAAGTT
Dilp 5	LEFT	5’- GCCTTGATGGACATGCTGA
	RIGHT	5’ - CATAATCGAATAGGCCCAAGG
Dilp 6	LEFT	5’- CCCTTGGCGATGTATTTCC
	RIGHT	5’- CACAAATCGGTTACGTTCTGC

Table 4: Effects of exposure to HESB on Drosophila Gene Expression

	$2^{-\Delta\Delta CT}$	
Gene	**Control**	**HESB**
CAT	1.14±0.6	2.71±0.72*
SOD	1.05±0.36	1.80±0.68
TrxR	1.02±0.30	2.59±0.36**
Nrf2	1.01±0.17	2.57±1.08**
Drice	0.84±0.43	4.68±0.72**
Dilp 2	1.00±0.12	0.93±0.13
Dilp 3	1.10±0.63	1.07±0.29
Dilp 5	1.01±0.24	1.13±0.66
Dilp 6	1.03±0.35	2.37±0.41*

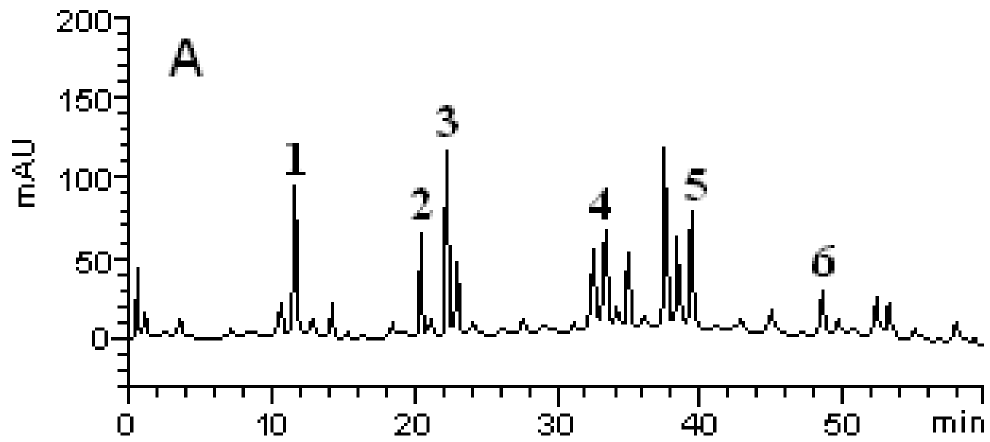
Data are expressed as mean ± SD of three determinations; *$p<0.05$, **$p<0.01$. Statistics were performed by Unpaired T test.

Table 5: Homology between *Drosophila melanogaster* and *Homo sapiens* genes

Drosophila melanogaster		***Homo sapiens***	
Name	**Symbol**	**Name**	**Symbol**
Catalase	Dmel\Cat	Catalase	CAT
Superoxide dismutase 1	Dmel\Sod1	Superoxide dismutase 1	SOD1
Superoxide dismutase 2	Dmel\Sod2	Superoxide dismutase 2	SOD2
Thioredoxin reductase 1	Dmel\Trxr-1	Thioredoxin reductase 1 Thioredoxin reductase 2	TRXR1 TRXR2
Thioredoxin reductase 2	Dmel\Trxr-2	Thioredoxin reductase 1 Thioredoxin reductase 2	TRXR1 TRXR2
Cap-n-collar	Dmel\cnc	Nuclear factor erythroid 2 like 1 Nuclear factor erythroid 2 Nuclear factor erythroid 2 like 2	NRF1 NFE2 NRF2
Drice (Death related ICE-like caspase)	Dmel\Drice	Caspase 7 Caspase 3	CASP7 CASP3
Dilp 2 (Insulin-like peptide 2)	Dmel\Ilp2	Insulin	INS
Dilp 3 (Insulin-like peptide 3)	Dmel\Ilp3	Insulin insulin like growth factor 1 insulin like growth factor 2	INS IGF 1 IGF 2
Dilp 5 (Insulin-like peptide 5)	Dmel\Ilp5	INS-IGF2 readthrough	INS-IGF2
Dilp 6 (Insulin-like peptide 6)	Dmel\Ilp6	Growth factor 1	IGF 1

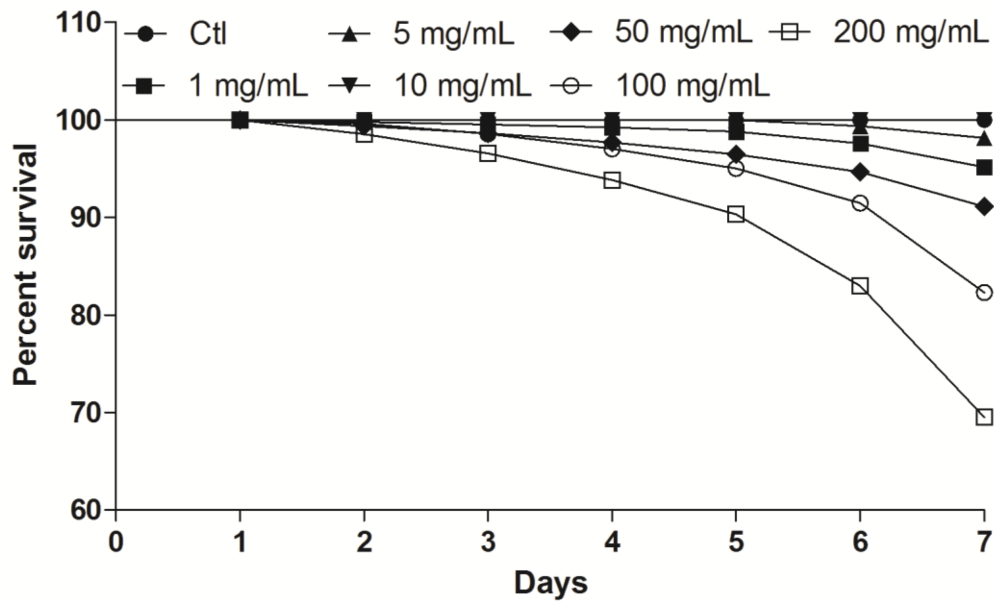
Fonte: Flybase in http://flybase.org/ , NCBI in https://www.ncbi.nlm.nih.gov/gene ,and Zhang et al (2009).

Figure 1



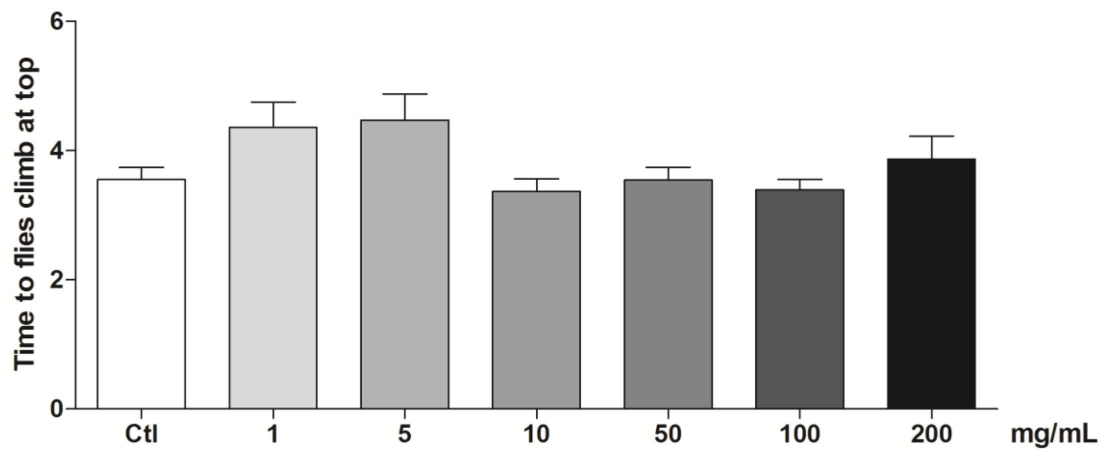
Representative high performance liquid chromatography profile of leaves of *Senecio brasiliensis* .Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), rosmarinic acid (peak 4), vitexin (peak 5), and quercetin (peak 6).

Figure 2



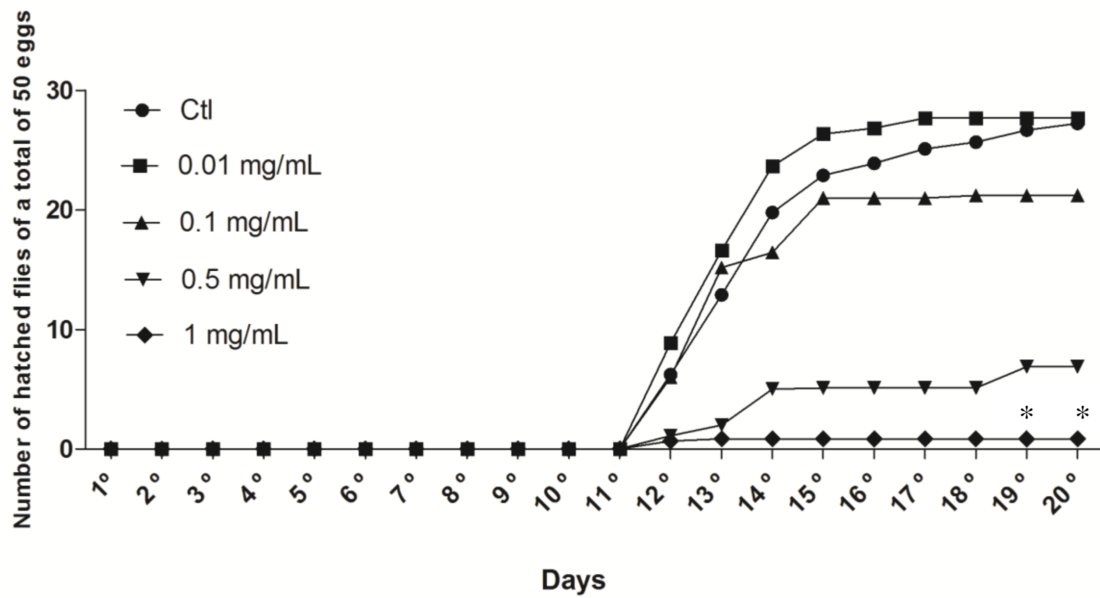
Effects of exposure to HESB on adult Drosophila survival. The survival rate was computed after flies were exposed to HESB at 1, 5, 10, 50, 100 and 200 mg/mL concentrations for 7 days. Experiments were performed individually and expressed as percentage of survived flies in relation to control group. Statistic was performed by comparing the survival curves with a log-rank (Mantel–Cox) test.

Figure 3



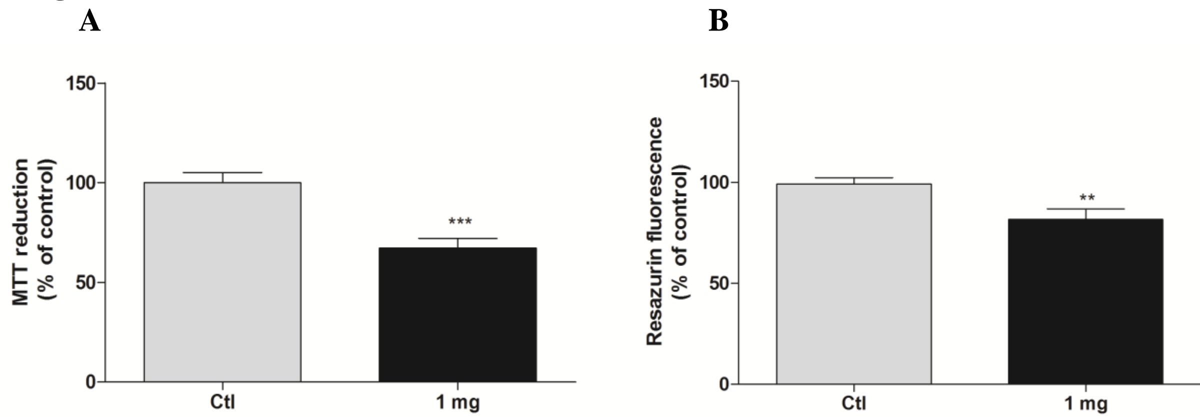
Effects of exposure to HESB on locomotor activity in adult *Drosophila melanogaster*. Individual negative geotaxis was performed after flies were treated with 0, 1, 5, 10, 50, 100 and 200 mg/mL concentrations of HESB for 7 days. Results are represented as mean ± standard error of the mean (SEM) climbing time of the flies. Statistic was performed by one-way ANOVA and Tukey's post hoc test.

Figure 4



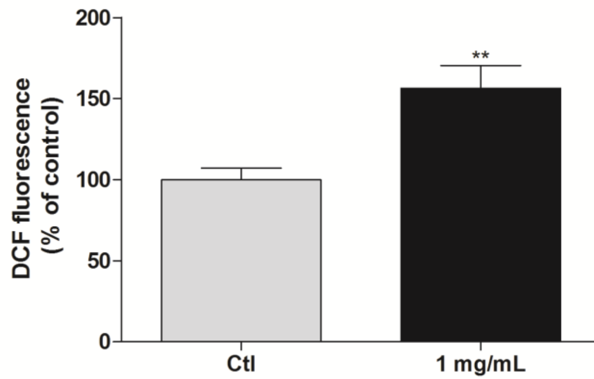
Effects of exposure to HESB during development of *Drosophila melanogaster*. The ecloded flies rate was computed after eggs, larvae, pupae and borned flies were exposed to HESB at 0.01, 0.1, 0.5 and 1 mg/mL concentrations for 20 days. Experiments were performed individually and are expressed as percentage of survived flies in relation to control group; *p<0.05. Statistic was performed by two-way ANOVA and Bonferroni post test.

Figure 5



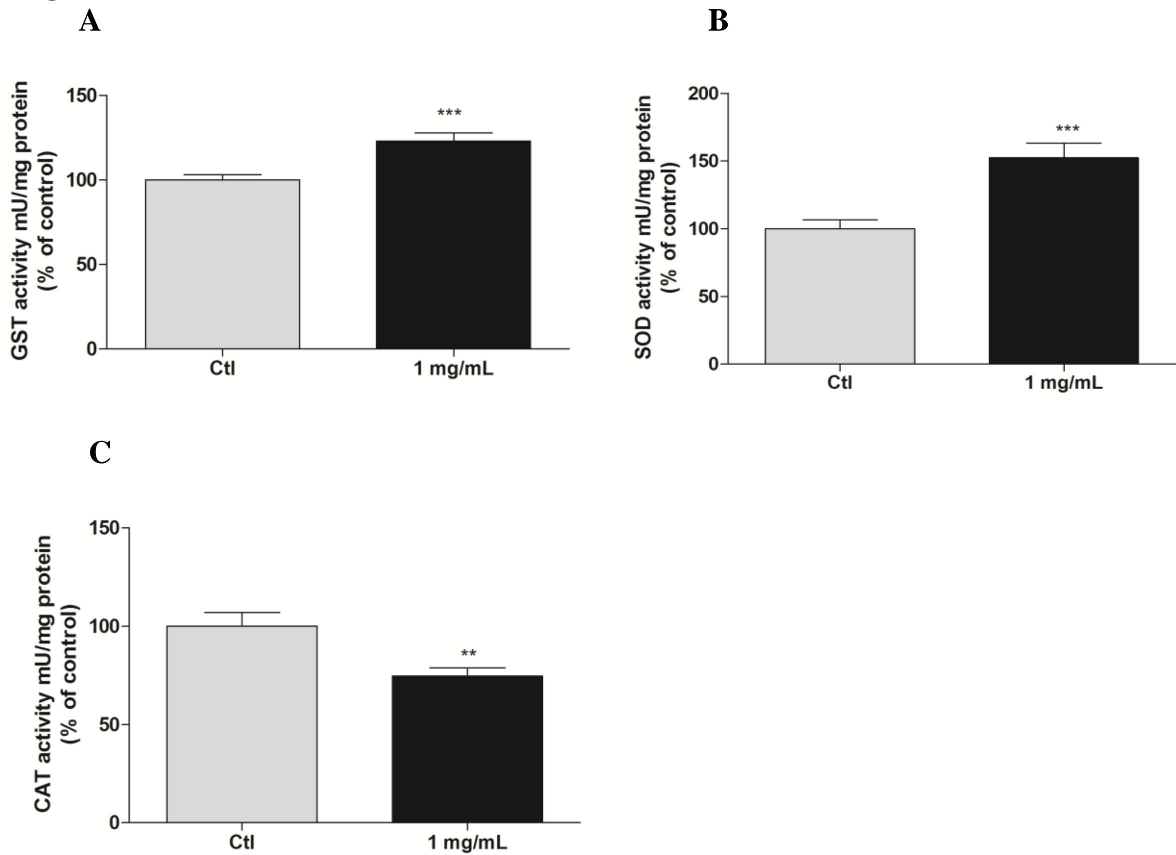
Effects of exposure to HESB on cellular viability in *Drosophila melanogaster* larvae. The evaluation of cellular viability was performed after larvae were treated with 0 and 1 mg/mL of HESB for 5 days by MTT absorbance (A) and Resazurin fluorescence (B) assays. Results are expressed as mean ± SEM and are expressed as percentage of control; **$p<0.01$; ***$p<0.001$. Statistical analysis was performed by Unpaired T test.

Figure 6



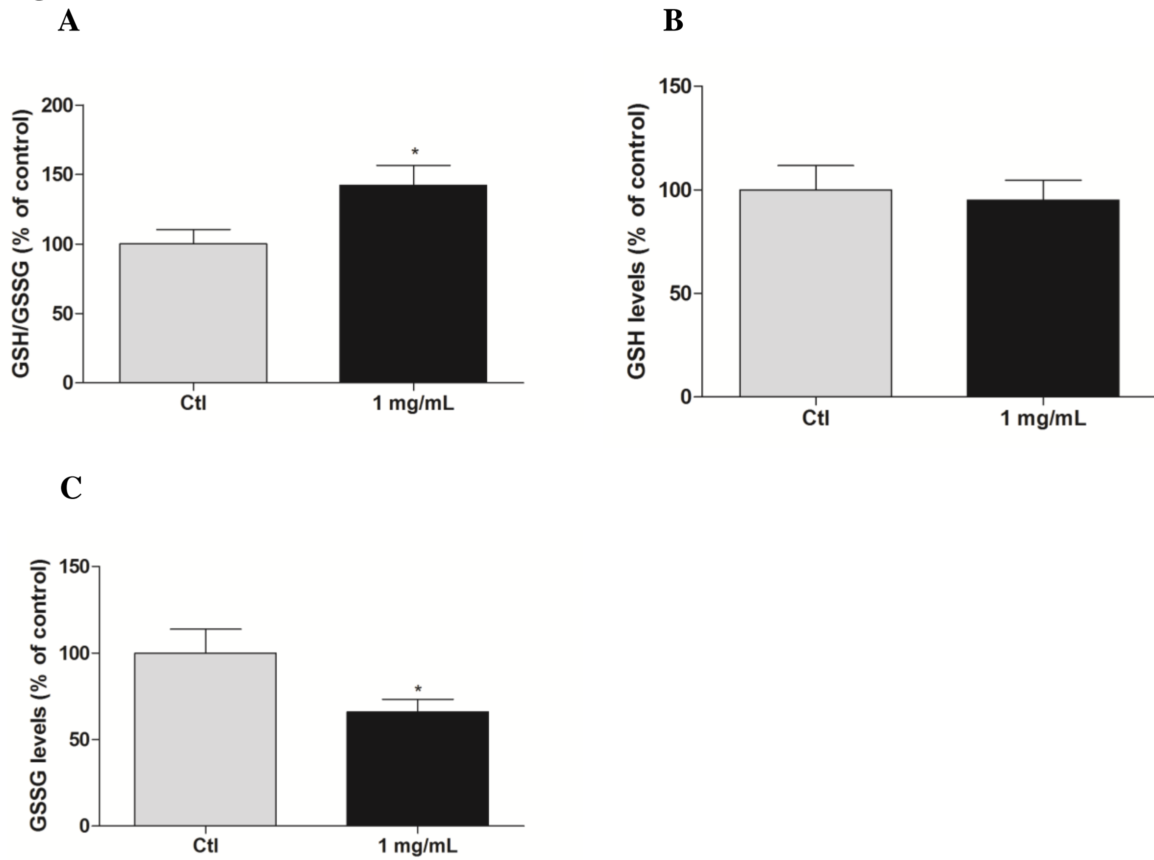
Effects of exposure to HESB on ROS production in *Drosophila melanogaster* larvae. The ROS generation index was measured after larvae were treated with 0 and 1 mg/mL of HESB for 5 days, as DCF-DA fluorescence. Results are expressed as mean ± SEM of raw fluorescence emitted following DCF-DA oxidation and in percentage of control; **$p<0.01$. Statistical analysis was performed by Unpaired T test.

Figure 7



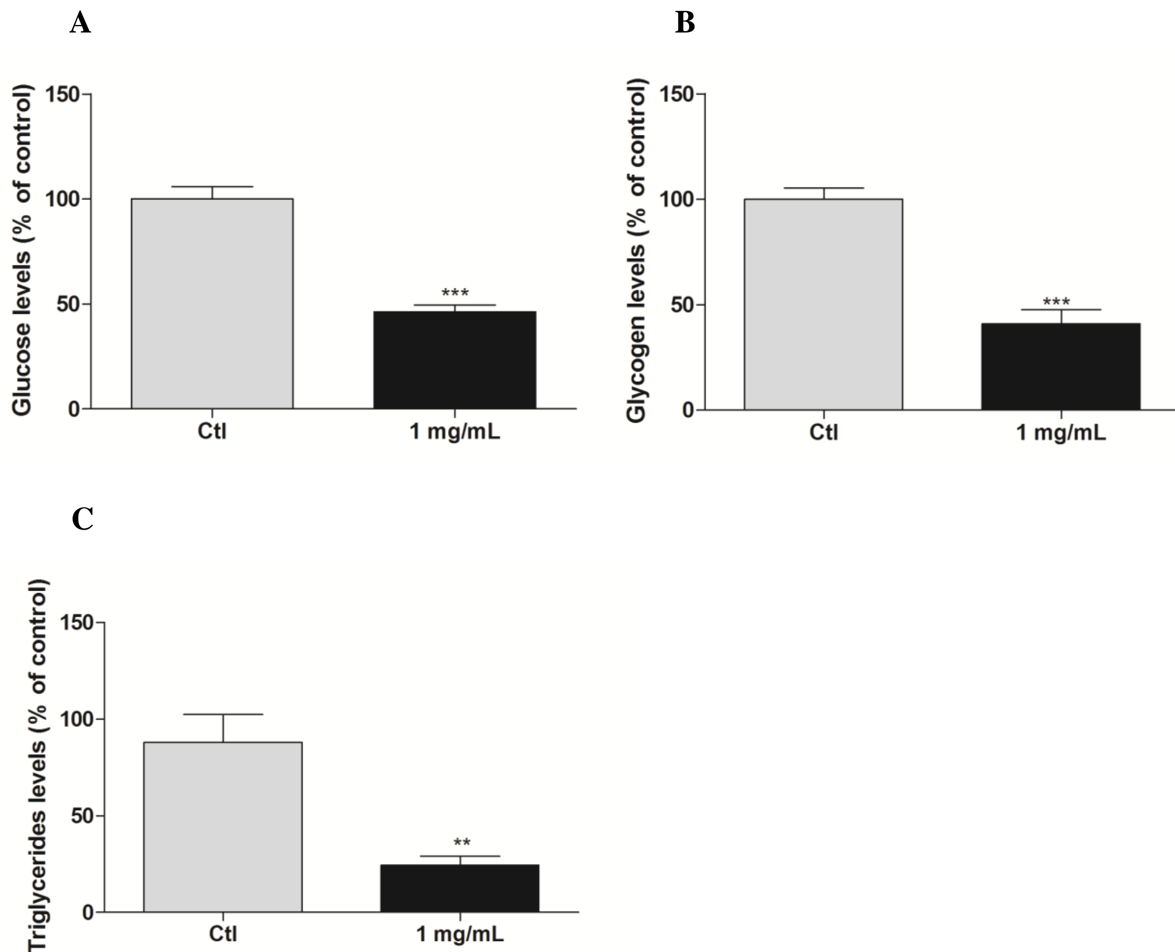
Antioxidant enzyme activity in *Drosophila melanogaster* larvae exposed to HESB. Larvae were treated with 0 and 1 mg/mL of HESB for 5 days, and glutathione s-transferase (GST) (A), superoxide dismutase (SOD) (B) and catalase (CAT) (C) activities were determined. Results are expressed as mean ± SEM and are expressed as percentage of control; $^{**}p<0.01$; $^{***}p<0.001$. Statistical analysis was performed by Unpaired T test.

Figure 8



Effects of exposure to HESB on glutathione levels in *Drosophila melanogaster* larvae. The evaluation of GSH/GSSG ratio (A), GSH (B) and GSSG (C) levels were performed after larvae were treated with 0 and 1 mg/mL of HESB for 5 days. Results are expressed as mean ± SEM and are expressed as percentage of control; *p<0.05. Statistical analysis was performed by Unpaired T test.

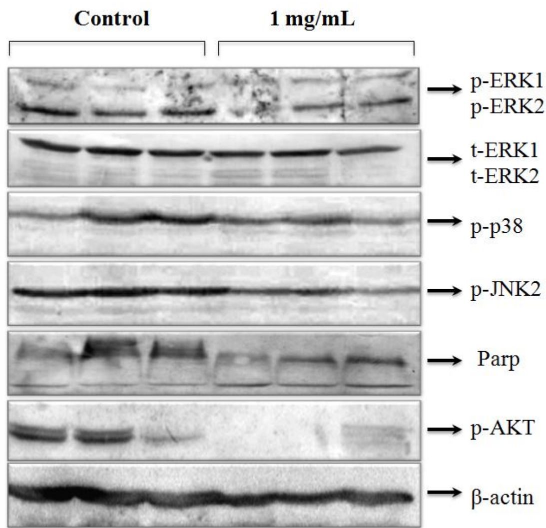
Figure 9



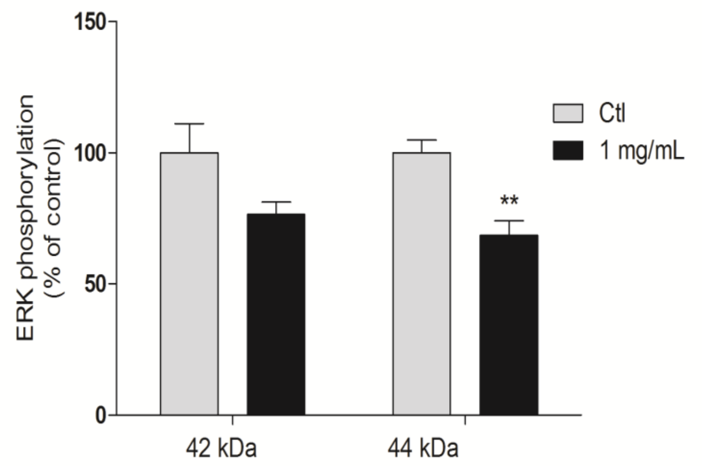
Effects of exposure to HESB on regulation of energetic metabolism in *Drosophila melanogaster* larvae. The evaluation of glucose (A), glycogen (B) and triglycerides (C) were performed after larvae were treated with 0 and 1 mg/mL of HESB for 5 days. Results are expressed as mean ± SEM and are expressed as percentage of control; **p<0.01; ***p<0.001. Statistical analysis was performed by Unpaired T test.

Figure 10

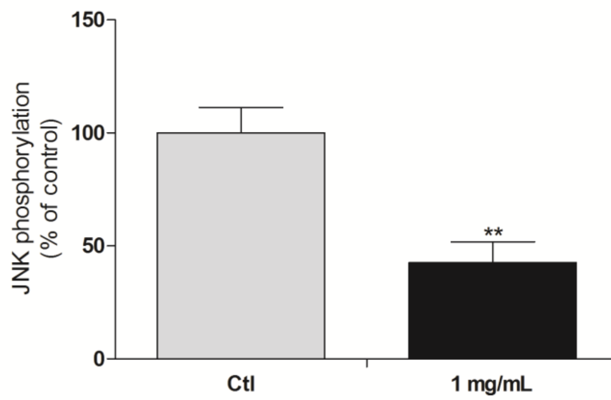
A



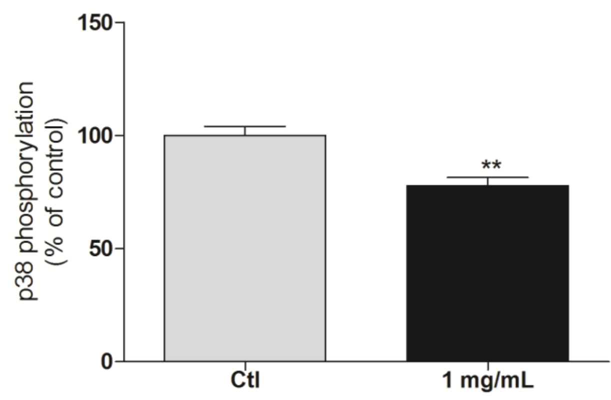
B



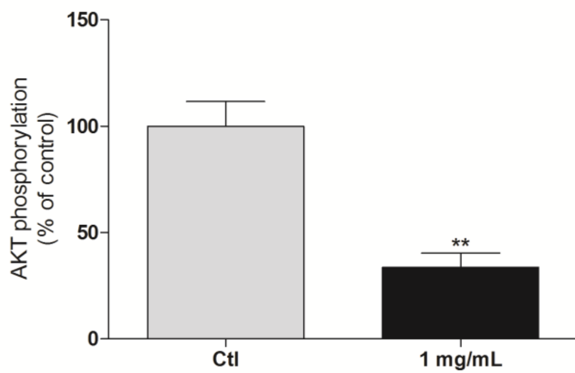
C



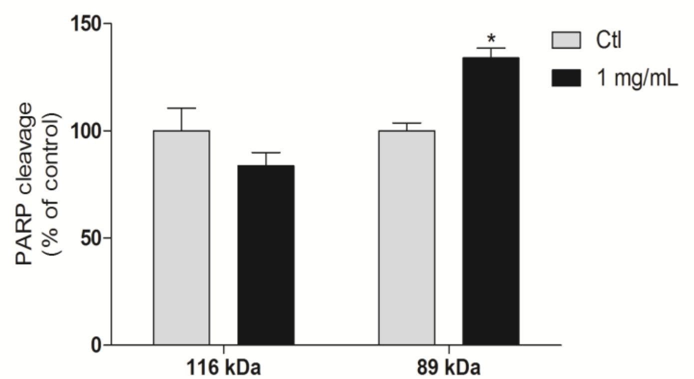
D



E

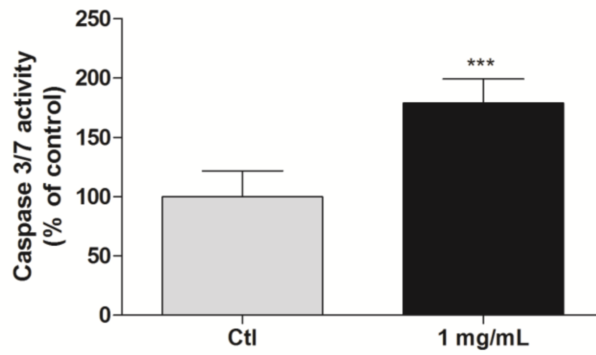


F



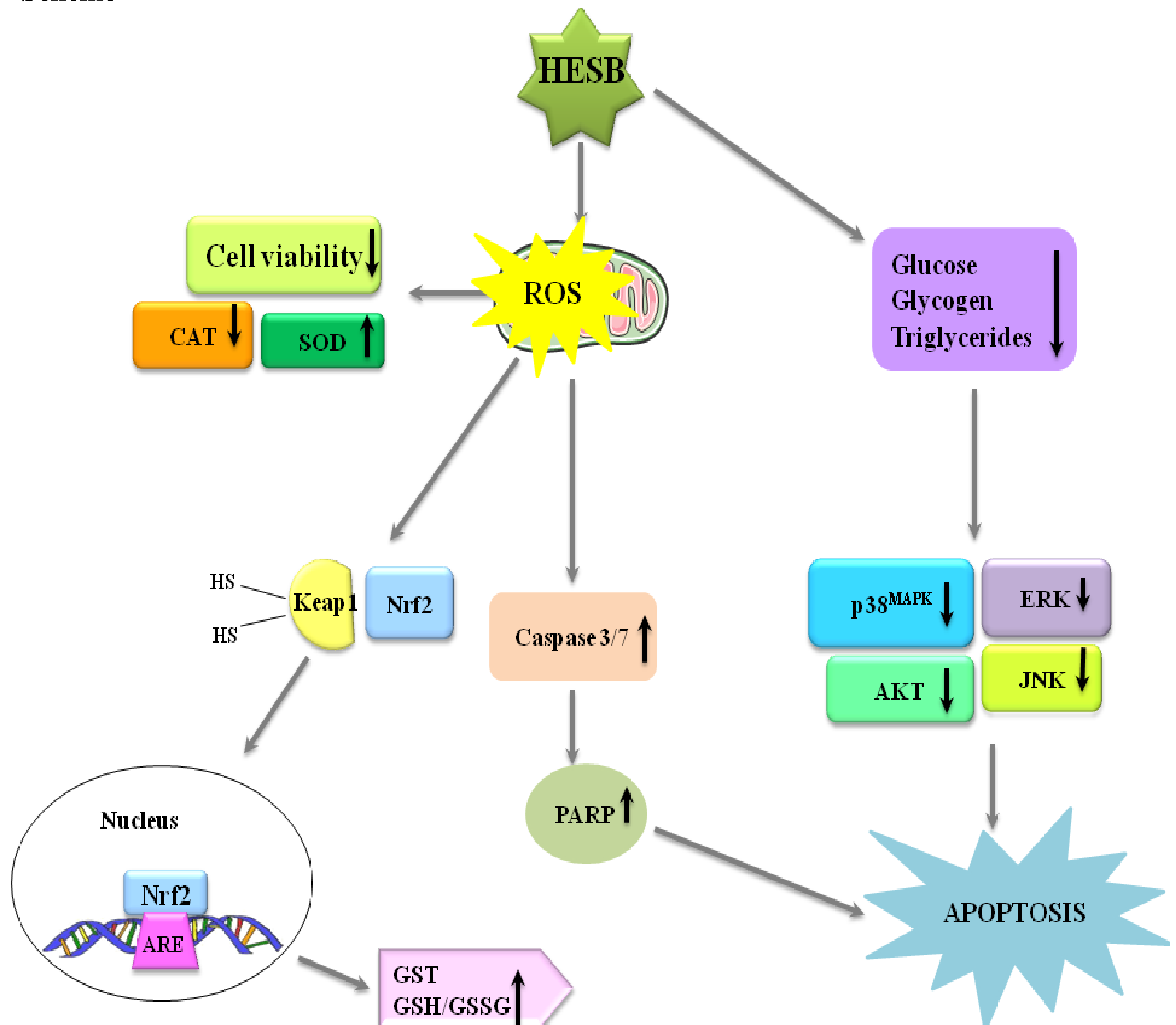
Effects of exposure to HESB on modulation of protein kinases and PARP cleavage in *Drosophila melanogaster* larvae. Modulation and phosphorylation of protein kinases and PARP cleavage were determined after larvae were treated with 0 and 1 mg/mL of HESB for 5 days. Representative Western blotting showing phosphorylated forms of MAPK ($p38^{MAPK}$, ERK1/2, JNK 1/2) AKT, PARP cleavage and total forms of ERK and β-actin (A). Protein kinases phosphorylation was normalized by β-actin expression (B-E), and PARP cleavage in relation to control (F). Results are represent as mean ± SEM of the densitometric quantification of immunoreactive bands and is expressed as the percentage of control; *$p<0.5$; **$p<0.01$. Statistics were performed by Unpaired T test.

Figure 11



Effects of exposure to HESB on caspases activity in *Drosophila melanogaster* larvae. Caspase 3/7 activity assay was performed after larvae were treated with 0 and 1 mg/mL of HESB for 5 days. Results are expressed as mean ± SEM and are expressed as percentage of control; ***$p<0.001$. Statistics were performed by Unpaired T test.

Scheme



Representative scheme for potentials mechanisms involved in the toxicity of HESB exposure on larvae *Drosophila melanogaster*. HESB constituents were able to induce ROS and lead to apoptosis. ROS decreased cellular viability and CAT activity, increasead SOD activity, and promoted the dissociation of Nrf2 from inhibitory protein Keap1. Nrf2 migrates for the nucleus and binds to the ARE, activating the transcription of detoxification metabolism pathways that involves GST and GSH. ROS also was able to induce caspases activation, caspase 3 cleaved and inactivated PARP, inducing apoptosis. ROS promoved by HESB constituints decreased ATP levels, which decreased glucose and glycogen levels and caused an inhibition of MAPK and AKT phosphorylation, due to lack of ATP, fact that also induced apoptosis.

5. Conclusões

Com base nos resultados obtidos pode-se verificar a toxicidade do extrato bruto das folhas de *S. brasiliensis* e sua capacidade de prejudicar o desenvolvimento e diminuir consideravelmente a taxa de eclosão de moscas adultas, podendo tal fato estar associado ao estresse oxidativo, indução da apoptose e inibição de proteínas associadas à sobrevivência celular. Dentre os principais dados obtidos neste estudo, lista-se:

- ✓ Diminuição da taxa de eclosão de moscas adultas;
- ✓ Diminuição da viabilidade das larvas;
- ✓ Aumento da atividade das enzimas GST e SOD e diminuição de CAT;
- ✓ Aumento da razão GSH/GSSG e da geração de ROS;
- ✓ Aumento da expressão dos genes Nrf2, TrxR, CAT, Drice e Dilp6;
- ✓ Diminuição da fosforilação das MAPK (ERK2, JNK1/2 e $P38^{MAPK}$) e AKT;
- ✓ Aumento da clivagem de PARP;
- ✓ Aumento da atividade das caspases 3/7;
- ✓ Diminuição dos níveis de glicose, glicogênio e triglicerídeos.

Assim sendo, os dados apresentados neste trabalho atentam para a importância em se conhecer as propriedades que as plantas utilizadas na medicina popular possuem e suas interações com os organismos, em vista dos danos que a sua toxicidade pode causar. *S. brasiliensis* demonstrou potencial de interromper a eclosão, levando a alterações fisiológicas em larvas de *D. melanogaster* que prejudicaram o seu desenvolvimento e afetaram profundamente a conclusão de seu ciclo de vida. Assim sendo, a importância deste estudo encontra-se na descoberta do potencial apoptótico e oxidante de *S. brasiliensis* em Drosophila, sendo que este fato pode ser expandido para células de mamíferos servindo como um agente terapêutico.

Referências

ALTIERI, M. **Biotecnologia agrícola: Mitos, riscos ambientais e alternativas**. Porto Alegre: Ascar-Emater, 2002.

ANDREOTTI, P F. **Interação de *Paracoccidioides brasiliensis* com células epiteliais. Caracterização de prováveis fatores de virulência**. Tese (Doutorado em Análises Clínicas) – Universidade Estadual Paulista, Araraquara, São Paulo, 2006.

BARBOSA, K B F et al. **Estresse oxidativo: conceito, implicações e fatores modulatórios,** Rev. Nutr., v.23, n. 4, p. 629-643, 2010.

BARREIROS, A L B S.; DAVID, J M.; DAVID, J P. **Estresse oxidativo: relação entre geração de espécies reativas e defesa do organismo,** Quim. Nova, v. 29, n. 1, p. 113-123, 2006.

BERKI, T et al. **Signal transduction (Medical Biotechnology).** 1 ed. Hungria: University of Pécs, 2011.

BETTIOL, W.; MORANDI, M. **Biocontrole de doenças de plantas: uso e perspectivas**. 1 ed. Jaguariúna: Embrapa meio ambiente, 2009.

BIODIVERSIDADE BRASILEIRA**. Avaliação e identificação de áreas e ações prioritárias para conservação, utilização sustentável e repartição dos benefícios da biodiversidade nos biomas brasileiros.** Brasília, 2002.

BIRSE, R T et al. **Regulation of insulin-producing cells in the adult *Drosophila* brain *via* the tachykinin peptide receptor DTKR,** J Exp Bio, v. 214, n. 24, p. 4201-4208, 2011.

BOLZANI, V S. **Biodiversidade, bioprospecção e inovação no Brasil**, Cienc. Cult, v. 68, n.1, p. 4-5, 2016.

BRIGHENT, A M. **Manual de identificação e manejo de plantas daninhas em cultivos de cana-de-açúcar.** 1 ed. Juiz de Fora: Embrapa Gado de Leite, 2010.

BRYAN, H K et al. **The Nrf2 cell defence pathway: Keap1-dependent and -independent mechanisms of regulation,** Biochem Pharmacol, v. 85, n. 6, p. 705–717, 2013.

CAMPOS, S C et al. **Toxicidade de espécies vegetais,** Rev. Bras. Pl. Med, v.18, n.1, p.373-382, 2016.

CHANG, F et al. **Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: a target for cancer chemotherapy,** Leukemia, v. 17, p. 590–603, 2003.

COGO, A J D et al. **Utilização de enzimas do estresse oxidativo como biomarcadoras de impactos ambientais**, Natureza on line, v. 7, n. 1, p. 37-42, 2009.

CORRÊA J C R.; SALGADO H R N. **Atividade inseticida das plantas e aplicações: revisão,** Rev. Bras. Plantas Med, v.13, n.4, p. 500-506, 2011.

CROZIER, A; CLIFFORD, M; ASHIHARA, H. **Plant secondary metabolites occurrence, structure and role in the human diet.** 1 ed. Ucrânia: Blackwell Publishing, 2006.

CZABOTAR, P E et al. **Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy**, Nat Rev Mol Cell Biol,v. 15, n. 1, p.49-63, 2014.

DOWNWARD, J. **Mechanism and consequences of activation of protein kinase B/Akt,** Current Opinion in Cell Biology, v. 10, n. 2, p. 262-267, 1998.

ELMORE, S. **Apoptosis: A review of programmed cell death,** Toxicol Pathol, v. 35, n. 4, p. 495–516, 2007.

FIRMO, W C A et al. **Contexto histórico, uso popular e concepção científica sobre plantas medicinais,** Cad. Pesq, v. 18, p. 90-95, 2011.

FLINT, M L.; BOSCH, R V D. **Introduction to integrated pest management.** 1 ed. New York: Plenum, 1981.

FU, P P et al. **Genotoxic pyrrolizidine alkaloids — mechanisms leading to DNA adduct formation and tumorigenicity,** Int. J. Mol. Sci, v. 3, n. 9, p. 948-964, 2002.

FUNK, V A et al. **Systematics, evolution, and biogeography of compositae.** 1 ed. Michigan: Hardcover, 2009.

FURTADO, R F et al. **Atividade larvicida de óleos essenciais contra *Aedes aegypti* L. (Diptera: Culicidae),** Neutr Entomol, v. 34, n. 5, p. 843-847. 2005.

GALLO, D et al. **Entomologia agrícola.** 1 ed. Piracicaba: FEALQ, 2002.

GEORGESCU, M-M et al, **PTEN tumor suppressor network in PI3K-Akt pathway control,** Genes Cancer, v.1, n. 12, p. 1170–1177, 2010.

GONZÁLEZ, C P et al. **Anti-inflammatory activity and composition of *Senecio salignus* Kunth**, BioMed Res Int, v. 2013, p. 1-5, 2013.

HASELTON, A T.; FRIDELL, Y-W C. **Adult *Drosophila melanogaster* as a model for the study of glucose homeostasis,** Aging, v. 2, n. 8, p. 523-526, 2010.

HAYAT, M. A. **Autophagy: cancer, other pathologies, inflammation, immunity, infection, and aging: v. 1. Molecular mechanisms**. 1ed. Nova Jersey: Elsevier, 2013.

HUAN, J-Y et al. **Species differences in the hepatic microsomal enzyme metabolism of the pyrrolizidine alkaloids,** Toxicol Lett, v. 99, n. 2, p. 127–137, 1998.

HUBER, P C.; ALMEIDA, W P. **Glutationa e enzimas relacionadas: papel biológico e importância em processos patológicos**, Quim. Nova, v. 31, n. 5, p. 1170-1179, 2008.

JENNINGS, B H. ***Drosophila* – a versatile model in biology & medicine,** Mat Today J, v. 14, n. 5, p. 190–195, 2011.

JOLY, C et al. **Diagnóstico da pesquisa em biodiversidade no Brasil.** Revista USP, n. 89, p. 114-33, 2011.

KANZOK, S M et al. **Substitution of the thioredoxin svstem for glutathione redudase in *Drosophila melanogaster***, Sci, v. 29, n. 5504, p. 643-646, 2001

KARAM, F S C et al. **Aspectos epidemiológicos da seneciose na região sul do Rio Grande do Sul Grande do Sul**, Pesq. Vet. Bras, v. 24, n. 4, p. 191-198, 2004.

KARAM, F S C.; SCHILD, A L.; BRAGA, J R M. **Intoxicação por *Senecio* spp. em bovinos no Rio Grande do Sul: condições ambientais favoráveis e medidas de controle,** Pesq. Vet. Bras, v. 31, n. 7, p. 603-609, 2011.

KAUFMANN, S H et al. **Specific proteolytic cleavage of poly(ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis,** Cancer Res, v 53, n. 17, p. 3976-3985, 1993.

KENNEDY, N J.; CELLURALE, C.; DAVIS, R J. **A radical role for p38 MAPK in tumor initiation, cancer cell,** v. 11, n. 2, p. 101-103, 2007.

KIM, E K.; CHOI, E-J. **Pathological roles of MAPK signaling pathways in human diseases,** Biochimica et Biophysica Acta, v. 1802, n. 4, p. 396–405, 2010.

KOURY, J C.; DONANGELO, C M. **Zinco, estresse oxidativo e atividade física**, Rev. Nutr, v 16, n. 4, p. 433-441, 2003.

KRINSKI, D.; MASSAROLI, A.; MACHADO, M. **Potencial inseticida de plantas da família Annonaceae,** Rev. Bras. Frutic, v. 36, p. 225-242, 2014.

KUMAR, S.; DOUMANIS, J. **The fly caspases,** Cell Death and Diff, v. 7, n. 11, p. 1039-1044, 2000.

LANGEL, D.; OBER, D.; PELSER, P B. **The evolution of pyrrolizidine alkaloid biosynthesis and diversity in the Senecioneae,** Phytochem Rev, v. 10, n. 1, p. 3–74, 2011.

LIZARRAGA, E et al. **Antioxidant, hemolytic and cytotoxic activities of Senecio species used in traditional medicine of Northwestern**, Nat Prod Commun., v. 7, n. 5, p. 607-608, 2012.

LOBO, V et al. **Free radicals, antioxidants and functional foods: Impact on human health**, Pharmacogn Rev, v. 4, n. 8, p. 118–126, 2010.

LOBODA, A et al. **Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism**, Cell. Mol. Life Sci, v. 73, n. 17, p. 3221–3247, 2016.

LONE, S H et al. **Essential oil composition of *Senecio graciliflorus* DC: comparative analysis of different parts and evaluation of antioxidant and cytotoxic activities,** Phytomedicine, v. 21, n. 6, p. 919-925, 2014.

LU, X.; TANG, K.; LI, P. **Plant metabolic engineering strategies for the production of pharmaceutical terpenoids,** Front Plant Sci, v. 7, n, 1647, p. 1-11, 2016.

MACEL, M.; VRIELING, K.; KLINKHAMER, P G.L. **Variation in pyrrolizidine alkaloid patterns of *Senecio jacobaea*,** Phytochem, v. 65, n. 7, p. 865–873, 2004.

MACIEL, M V et al. **Extratos vegetais usados no controle de dípteros vetores de zoonoses,** Rev. Bras. Pl. Med., Botucatu, v.12, n.1, p.105-112, 2010.

MAGNABOSCO, E M et al. **Hepatic veno-oclusive disease: Report of case,** Journal of Pediatrics, Nova York, v. 73, n. 2, p. 115-118, 1997.

MANUBOLU, M et al. **Activity-guided isolation and identification of anti-staphylococcal components from *Senecio tenuifolius* Burm. F. leaf extracts**, Asian Pac J Trop Biomed, v. 3, n. 3, p. 191–195, 2013.

MARANGONI C.; MOURA N F.; GARCIA F R M. **Utilização de óleos essenciais e extratos de plantas no controle de insetos**, Rev. Ciênc. Amb, v. 6, n. 2, p. 95-112, 2012.

MARQUES, Y M F S**. Análise da Via Akt em neoplasias benignas e malignas de glândulas salivares.** 84 f. Tese (Doutorado em Odontologia) – Faculdade de Odontologia da Universidade de São Paulo, São Paulo, São Paulo, 2010.

MATZENBACHER, N I. **Uma nova espécie do gênero *Senecio* L. (Asteraceae - Senecioneae) no Rio Grande do Sul, Brasil,** Iheringia, v. 64, n. 1, p. 109-113, 2009.

MCILWAIN, D R; BERGER, T.; MAK, T W. **Caspase functions in cell death and disease,** Cold Spring Harb Perspect Biol, v. 64, n. 1, p. 109-113, 2013.

MOJZER, E B et al. **Polyphenols: extraction methods, antioxidative action, bioavailability and anticarcinogenic effects,** Molecules, v. 21, n. 7, p. 1-38, 2016.

MOLLINEDO, P et al. **Anti-infective assessment of *Senecio smithioides* (Asteraceae) and isolation of 9-oxoeuryopsin, a furanoeremophilane-type sesquiterpene with antiplasmodial activity,** Nat Prod Res, v. 30, n. 22, p. 2594- 2597, 2015.

MORALES, J C et al. **Review of Poly (ADP-ribose) Polymerase (PARP) mechanisms of action and rationale for targeting in cancer and other diseases,** Crit Rev Eukaryot Gene Expr, v. 24, n. 1, p. 15–28, 2014.

MORALES, M M. **Métodos alternativos à utilização de animais em pesquisa científica: Mito ou realidade?** Cienc. Cult, v. 60, n. 2, p. 33-36, 2008.

MOREIRA, H J C.; BRAGANÇA, H B N. **Manual de identificação de plantas infestantes Hortifrúti**. 1 ed. Campinas: FMC Agricultural Products, 2011.

MORÓN, U M.; CASTILLA-CORTÁZAR, I. **Protection against oxidative stress and IGF-I deficiency conditions.** In: EL-MISSIRY, M A. Antioxidant Enzyme. Croatia: InTech, 2012. p. 90

NELSON, D L.; COX, M M. **Princípios de Bioquímica de Lehninger**. 6 ed. Porto Alegre: Artmed, 2014

OGA, S., CAMARGO, M M A.; BATISTUZZO, J A O. **Fundamentos de Toxicologia**. 3 ed. São Paulo: Athemeu editora, 2008.

OLIVEIRA, C C et al**. Senecio L. (Asteraceae, Senecioneae) no Estado do Rio de Janeiro, Brasil,** Hoehnea journal, v. 42, n. 4, p. 703-724, 2015.

OSBRURN, W O.; KENSLER, T W. **Nrf2 signaling: an adaptive response pathway for protection against environmental toxic insults**, Mutat Res, v. 659, n. 1-2, p. 31-39, 2008.

PANDEY, U.; NICHOLS, C. **Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery**, Pharm Reviews, v. 63, n. 2, p. 411–36, 2011.

PHAM-HUY, L A.; HE, H.; PHAM-HUY, C. **Free radicals, antioxidants in disease and health**, Int J Biomed Sci, v. 4, n. 2, p. 89–96, 2008.

PINTO, A et al. **Produtos naturais: atualidade, desafios e perspectivas,** Quim Nova, v. 25, n. 1, p. 45- 61, 2002.

PONCIO, S. **Bioatividade de inseticidas botânicos sobre *Microtheca ochroloma* Stal (Coleoptera: Chrysomelidae)**. 80 f. Dissertação (Mestrado em Agronomia) – Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, 2010.

PRAKASH, A S et al. **Pyrrolizidine alkaloids in human diet**, Mutat Res, v. 443, n. 1-2, p. 53-67, 1999.

RAY, P D.; HUANG, B-W.; TSUJI, Y. **Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling,** Cell Signal, v. 24, n. 5, p. 981–990, 2012.

RIBEIRO, L P et al. **Effect of botanical insecticides on the attractiveness and oviposition preference of *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) in tomato**, Rev. Bras. De Agroecologia, v. 4, n. 2, p. 2009.

ROEL, A. **Utilização de plantas com propriedades inseticidas: Uma contribuição para o desenvolvimento rural sustentável**, Rev Int Des Loc, v. 1, n. 2, p. 43-50, 2001.

SALVESEN, G.S.; DIXIT, M.V. **Caspase activation: The induced-proximity model,** Proc. Natl. Acad. Sci, v. 96, n. 20, p. 10964–10967, 1999.

SANDINI, T M et al. ***Senecio brasiliensis* e alcaloides pirrolizidínicos: Toxicidade em animais e na saúde humana,** Biot, v. 26, n. 2, p. 83-92, 2013.

SANTOS, J C A et al. **Patogênese, sinais clínicos e patologia das doenças causadas por plantas hepatotóxicas em ruminantes e eqüinos no Brasil**, Pesq. Vet. Bras, v. 28, n. 1, p. 1-14, 2008.

SCANGA, S E et al. **The conserved PI3'K/PTEN/Akt signaling pathway regulates both cell size and survival in Drosophila**, Oncog, v. 19, n. 35, p. 3971-3977, 2000.

SCHNEIDER, C D.; Oliveira; A R. **Radicais livres de oxigênio e exercício: Mecanismos de formação e adaptação ao treinamento físico,** Rev Bras Med Esporte, v. 10, n.4, p. 314-318, 2004.

SCHREIBER, V et al. **Poly (ADP-ribose): Novel functions for an old molecule**, Mol Cell Bio, v. 7, p. 517-528, 2006.

SCULLY, L R.; BIDOCHKA, M J. **Developing insectmodels for the study of current and emerging human pathogens**, FEMS Microbiol Lett, v. 263, n. 1, p. 1-9, 2006.

SHINGLETON, A W. **The regulation of organ size in Drosophila physiology, plasticity, patterning and physical force**, Organog, v. 6, n. 2, p. 76-87, 2010.

SILVA, B Q.; HAHN, S R. **Uso de plantas medicinais por indivíduos com hipertensão arterial sistêmica, Diabetes mellitus ou dislipidemias**, R. Bras. Farm. Hosp. Serv. Saúde São Paulo, v. 2, n. 3, p. 36-40, 2011.

SILVA, B V et al. **Proteínas quinases: Características estruturais e inibidores químicos,** Quim. Nova*,* v. 32, n. 2, p. 453-462, 2009.

SILVA, C M.; BOLZAN, A A.; HEINZMANN, B M. **Alcalóides pirrolizidínicos em espécies do gênero *Senecio***, Quim. Nova, v. 29, n. 5, p. 1047-1053, 2006.

SMITH, L W.; CULVENOR, C C J. **Plant sources of hepatotoxic pyrrolizidine alkaloids***,* J. Nat. Prod, v. 44, n. 2, p. 129–152, 1981.

SON, T G.; CAMANDOLA, S.; MATTSON, M P. **Hormetic dietary phytochemicals,** Neuromolecular Med, v. 10, n. 4, p. 236-246, 2008.

SONG, G.; OUYANG, G.; BAO, S. **The activation of Akt/PKB signaling pathway and cell survival,** J. Cell. Mol. Med, v. 9, n. 1, p. 59-71, 2005.

SOUZA, R R et al. **Modulatory effect of *Senecio brasiliensis* (Spreng) Less. in a murine model of inflammation induced by carrageenan into the pleural cavity**, Journal of Ethnopharmacology, v. 168, p. 373–379, 2015.

STRANGE, K. **Drug discovery in fish, flies, and worms,** ILAR Journal, v. 57, n. 2, p. 133–143, 2016.

TAIZ, L.; ZEIGER, E. **Fisiologia Vegetal**. 3ed. São Paulo: Artmed, 2006.

TANIGUCHI, A N R et al. **Relato de caso – doença veno-oclusiva induzida por chá de *Senecio brasiliensis*.** Revista do Hospital de Clínicas de Porto Alegre, v. 22, n. 1, p. 183-184, 2002.

TEKMAN, B et al. **Purification and characterization of glutathione reductase from rainbow trout (*Oncorhynchus mykiss*) liver and inhibition effects of metal ions on enzyme activity,** Comp Biochem Physiol C Toxicol Pharmacol, v. 148, n. 2, p. 117–121, 2008.

TELES, A M. *Senecio* in **Lista de Espécies da Flora do Brasil**. Jardim Botânico do Rio de Janeiro. 2015. Disponível em: <http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB115655>. Acesso em: 01 Ago. 2016).

TERNES, A P L et al. ***Drosophila melanogaster* – an embryonic model for studying behavioral and biochemical effects of manganese exposure**, EXCLI Journal, v. 13, p. 1239–1253, 2014.

TEWARI, M et al. **Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase,** Cell, v. 81, n. 5, p.801-809, 1995.

THACKER, J R M. **An introduction to arthropod pest control.** 1 ed. Cambridge: Cambridge University Press, 2002.

TIDJANI, S et al. **Analysis of pyrrolizidine alkaloids and evaluation of some biological activities of Algerian *Senecio delphinifolius* (Asteraceae),** Nat Prod Commun, v. 8, n. 4, p. 439-440, 2013.

TOMA, W et al. **Modulation of gastrin and epidermal growth factor by pyrrolizidine alkaloids obtained from *Senecio brasiliensis* in acute and chronic induced gastric ulcers**, Can J Physiol Pharmacol, v. 82, n. 5, p.319-325, 2004.

VICKERY, M L.; VICKERY, B. **Secondary plant metabolism.** 1 ed. Hong Kong: The Macmillan Press Ltd, 1981.

XIAO, K-J et al. **Anti-inflammatory activity and chemical composition of the essential oils from *Senecio flammeus*,** EXCLI Journal, v. 18, n. 13, p. 782-791, 2014.

XU, D et al. **Genetic control of programmed cell death (apoptosis) in Drosophila**, Landes Bioscience, v. 3, n. 1, p. 78-90, 2009.

ZAROGOULIDIS, P et al. **mTOR pathway: A current, up-to-date mini-review (Review),** Oncol Lett, v. 8, n. 6, p. 2367–2370, 2014.

ZHANG, D D. **Mechanistic studies of the Nrf2-Keap1 signaling pathway,** Drug Metab Rev, n. 38, v. 4, p. 769-789, 2006.

ZHANG, H et al. **Deletion of Drosophila insulin-like peptides causes growth defects and metabolic abnormalities**, Proc Natl Acad Sci U S A, v. 106, n. 46, p. 19617–19622, 2009.

ZHANG, M et al. **Emerging roles of Nrf2 and phase II antioxidant enzymes in neuroprotection,** Progress in Neurobiology, v. 100, p. 30-47, 2013

ZUCKER, S N et al. **Nrf2 amplifies oxidative stress via induction of Klf9,** Mol Cell, v. 53, n. 6, p. 916–928, 2014.