UNIVERSIDADE FEDERAL DE SANTA MARIA

CENTRO DE CIÊNCIAS NATURAIS E EXATAS

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA TOXICOLÓGICA – PPGBTOX

Artemísia absinthium MINIMIZA A TOXICIDADE INDUZIDA POR PARACETAMOL EM CAMUNDONGOS

Fernando Wendel Franco

Santa Maria, 2015.

Artemísia absinthium MINIMIZA A TOXICIDADE INDUZIDA POR PARACETAMOL EM CAMUNDONGOS

Fernando Wendel Franco

Dissertação Apresentada ao Curso de Mestrado do Programa de Pós -Graduação em Bioquímica Toxicológica da Universidade Federal de Santa Maria (UFSM, RS) como requisito parcial para a obtenção do grau de **Mestre em Bioquímica**

Gustavo Orione Puntel

Santa Maria, RS, Brasil

2015

Universidade Federal de Santa Maria

Centro de Ciências Naturais e Exatas

Programa de Pós Graduação em Ciências Biológicas:

Bioquímica Toxicológica

A Comissão Examinadora, abaixo assinada, aprova a Dissertação de Mestrado

***Artemísia absinthium* MINIMIZA A TOXICIDADE INDUZIDA POR PARACETAMOL EM CAMUNDONGOS**

elaborada por

Fernando Wendel Franco

como requisito parcial para a obtenção do grau de **Mestre em Ciências Biológicas: Bioquímica Toxicológica**

COMISSÃO EXAMINADORA:

Gustavo Orione Puntel, DR. (UFSM)

(Presidente/Orientador)

Liliane de Freitas Bauermann (UFSM)

Thiago Henrique Lugokenski, DR. (UNIPAMPA)

“O cientista não é o homem que fornece as verdadeiras respostas; é quem faz as verdadeiras perguntas”.

(Claude Lévi-Strauss)

AGRADECIMENTOS

Primeiramente agradeço aos meus familiares, pelo amor, confiança e pelo apoio em todos os momentos. Vocês são os meus exemplos, minhas referências e também meu porto seguro. Tudo o que conquistei e sou hoje é porque sempre tive vocês ao meu lado. As conquistas não são apenas minhas, mas de vocês também.

Aos Prof. Gustavo Orione Puntel, Félix Soares e Nilda de Vargas Barbosa pelas orientações, pela disponibilidade de tempo, por terem compartilhado seus conhecimentos e por todo apoio na minha vida acadêmica.

Aos Colegas de laboratório: Gerson Torres, Guilherme Pires Amaral, Sílvio Terra Stefanello, Diane Duarte Hartmann, Rodrigo Pereira Martins. Agradeço pela amizade, pela parceria, por dividirem seus conhecimentos, pelas muitas risadas, pela ajuda e por todo apoio.

Ao Prof. João Batista e ao seu laboratório, agradeço pela atenção em todos os momentos em que precisei de seus conhecimentos. Aos demais professores, colegas e funcionários deste Programa de Pós Graduação, agradeço a disposição para me ajudar e a contribuição, de alguma forma, para a realização de meu trabalho e para a minha formação.

Por fim, agradeço a Universidade Federal de Santa Maria e ao Programa de Pós Graduação em Bioquímica Toxicológica pela possibilidade de realização deste curso.

RESUMO

Dissertação de Mestrado

Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica

Universidade Federal de Santa Maria, RS, Brasil

Artemísia absinthium MINIMIZA A TOXICIDADE INDUZIDA POR PARACETAMOL EM CAMUNDONGOS

AUTOR: FERNANDO WENDEL FRANCO

ORIENTADOR: GUSTAVO ORIONE PUNTEL

O Paracetamol (APAP) é um analgésico muito popular associado às ingestões tóxicas. Há muitos estudos sobre a prevenção da hepatotoxicidade induzida por APAP usando plantas medicinais. Porém muito poucos utilizando a Artemisia absinthium (AA) popularmente conhecida como losna. Este estudo tem o objetivo de investigar os efeitos antioxidantes do extrato aquoso de AA contra a intoxicação por APAP em camundongos. Os resultados mostram que o extrato de AA apresenta grandes concentrações de polifenóis e flavonóides. Em testes *in vitro*, houve uma diminuição acentuada na peroxidação e, a atividade quelante de radical DPPH foi reduzida nas concentrações crescentes de extrato. Posteriormente, *ex vivo,* AA reduziu a peroxidação lipídica induzida por ferro. Na fase de intoxicação com APAP houve um aumento significativo na peroxidação lipídica e também uma diminuição dos níveis de tiol não proteico e das enzimas superóxido dismutase e catalase nos tecidos hepático, renal e cerebral. O APAP causou um aumento das atividades de enzimas de transaminases no soro (ALT e AST), e também diminuiu a viabilidade celular. Em geral, esses efeitos podem estar relacionados com o seu potencial antioxidante *in vitro* e *ex vivo*. Ainda, mais estudos são necessários para destacar o mecanismo de ação do extrato aquoso de AA, especialmente seus efeitos na prevenção de disfunção mitocondrial. Estes resultados mostram que o extrato aquoso de AA tem propriedades hepato protetoras contra a toxicidade induzida por APAP.

Palavras-chave: hepatotoxicidade, acetaminofeno, antioxidante, Artemisia absinthium.

ABSTRACT

Master Dissertation
Graduation Program in Biological Sciences: Toxicological Biochemistry
Federal University of Santa Maria, RS, Brazil

Artemísia absinthium MINIMIZE ACETAMINOPHEN INDUCED TOXICITY IN MICE

AUTHOR: FERNANDO WENDEL FRANCO

ADVISOR: GUSTAVO ORIONE PUNTEL

Acetaminophen is a popular analgesic associated with toxic ingestions. There are many studies about prevention of APAP induced hepatotoxicity including the medicinal plants. *Artemisia absinthium* (AA) is one of them, but its extract against the hepatotoxicity induced by APAP were not yet investigated. This study aimed to investigate the antioxidant effects of AA's aqueous extract against the APAP's intoxication in mice. Our results show that AA present high poliphenol's concentrations and flavonoids. In vitro tests, AA decreased the basal and pro-oxidants induced lipid peroxidation in mice homogenated tissues (10 to 25µg). Also, DPPH radical's (2,2-diphenyl-1-picrylhydrazyl) scavenger activity was reduced (100µg) ($p \leq 0.05$). *Ex vivo*, AA reduced the lipid peroxidation, induced by iron reduced, in mice liver (from 10 to 100µg) and kidneys (at 100µg) ($p \leq 0.05$). The intoxication with APAP determined a significant increase in lipid peroxidation and also a decrease in non-protein thiol levels and of the enzymes superoxide dismutase e catalase activities ($p \leq 0.05$). Moreover, the APAP depicted an increase of the transaminase enzymes activities in serum, and also decreased the cell viability in liver and brain's mice ($p \leq 0.05$). Our results show that the aqueous extract of AA had hepatoprotective properties against toxicity induced by APAP. In general, the hepatoprotective effects of AA against an intoxication with APAP were related to its antioxidant potential *in vitro* and *ex vivo.* Finally, further studies are needed to highlight the mechanism of AA aqueous extract action, especially its effects in the mitochondrial dysfunction prevention.

Key Words: hepatotoxicity, acetaminophen, antioxidant, Artemisia absinthium

LISTA DE TABELAS:

LISTA DE FIGURAS:

LISTA DE ABREVIATURAS

AA- ArtemísiaAbsínthium

AINES- Antinflamtórios não esteroidais

ALT -Alanina amino transferase

APAP- Paracetamol

AST- Aspartato amino transferase

CAT- Catalase

DFCH-DA- Diclorofluorceína oxidada

DPPH- 2,2-difenil-1-picril-hidrazila

MTT - (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)

NAPQI- N-acetil-p-benzo-quinona imina

NPSH –non protein thiols

PGE- Prostaglandina Endoperóxido Sintase

SOD-Superóxido Dismutase

TBARS- ThioBarbituric Acid Reactive Substances

SUMÁRIO

INTRODUÇÃO

1.1 O Paracetamol

O Acetaminofeno (N-acetil-p-aminofenol), também conhecido como paracetamol (APAP) é um analgésico popular e largamente utilizado. É descrito como um fármaco associado a ingestões tóxicas em tentativas de suicídio ou quando não usado na dose correta. O APAP é classificado como um anti-inflamatório não esteroidal (AINE) apesar de não apresentar a mesma atividade anti-inflamatória que os demais fármacos da classe. Ele também é notado por não causar efeitos colaterais gastrointestinais acentuados, como os demais AINES. (Bertolini et al. 2006).

O APAP é um composto p-aminofenólico. Os derivados p-aminofenólicos são compostos de síntese derivados da anilina (paracetamol e fenacetina), que se distinguem pelos seus efeitos tóxicos. Compostos como o APAP são sintetizados a partir de substituições eletrofílicas em aromáticos como descrito na fórmula estrutural da Figura 1. (Vollhard et al., 2007).

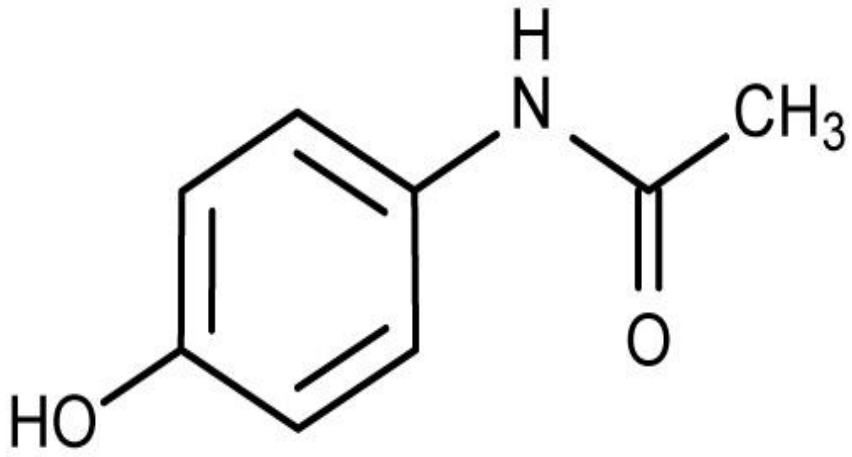

Fig 1. Estrutura Química do APAP

1.1.1 Ação farmacológica

Farmacologicamente, o APAP é utilizado devido às suas ações: analgésica, antipirética e antinflamatória. O fármaco apresenta-se disponível em várias formas de apresentação e concentrações. Pode ser adquirido de forma isolada ou em associações.

A apresentação pode ser na forma de comprimidos, cápsulas ou soluções de uso oral. A concentração do fármaco varia conforme a apresentação, como soluções de 100 ou 200mg/ml, comprimidos ou cápsulas de 500 ou 750mg, dentre outras, bem como pela presença ou não de outras fórmulas associadas (Leung et al. 1992).

O APAP é um substituto adequado da aspirina para uso analgésico ou antipirético. O uso crônico de doses de até 2g/dia não está associado à disfunção hepática. Ele tem particularmente um grande valor para os pacientes nos quais a aspirina não é indicada. Embora seja indicado para pacientes com osteoartrites não inflamatórias, por exemplo, ele não é tão eficaz como a aspirina ou outros antiinflamatórios não esteroidais no tratamento de artrites (Leung et al. 1992).

Sabe-se que o APAP atua na inibição da enzima cicloxigenase 2 (COX-2). Este efeito por sua vez é inibido na presença de grandes concentrações de peróxidos. Como o sistema nervoso central apresenta baixas concentrações de peróxidos, estariam assim justificados os seus efeitos analgésicos e antipiréticos do APAP no sistema nervoso central. Consequentemente seu fraco efeito antiinflamatório estaria explicado pelos elevados níveis de peróxidos nos demais sítios inflamatórios (Kis et al. 2005).

A COX inicialmente relacionada com a ação do APAP no sistema nervoso central foi denominada COX-3. Acreditava- se que esta fosse uma variante das COX-1 e COX-2. Essa hipótese surgiu após ser descoberta uma nova COX no cérebro de caninos. Atualmente as pesquisas não apresentam nenhuma evidencia concreta da existência de um terceiro gene independente para a COX (Kis et al. 2005).

A inibição da COX por APAP foi sugerida devido a capacidade deste em extinguir o radical tirosil presente na estrutura molecular da Prostaglandina-Endoperóxido Sintase (PGE). Além disso, compostos análogos ao APAP, porém monometilados também se mostraram capazes de inibir as PGEs. Outros compostos análogos contendo átomos de flúor adjacentes ao grupo hidroxila, adjacentes ao grupamento amida ou ainda ao grupo acetamida inibiram a COX em ensaios realizados *in vivo* e *in vitro.* (Barnard et al.,1993).

1.1.2. Toxicocinética do paracetamol

1.1.2.1. Absorção

A absorção do paracetamol após ser administrado por via oral depende do tempo de esvaziamento gástrico. Posteriormente ele é absorvido no intestino delgado de forma rápida e total. O pico de concentração plasmática é atingido em cerca de 30 a 60 minutos e sua meia vida é de cerca de 2 horas. Dados esses considerados após a ingesta de doses terapêuticas. Tais valores são prolongados nas overdoses, havendo uma correlação direta com a dose ingerida (Goodman et al., 2003).

O fígado entra em contato com a maior parte dos nutrientes e outras substâncias exógenas como o APAP. Já no intestino, essas substancias são absorvidas e então distribuídas para os demais tecidos. Esta característica da absorção oral é chamada de efeito ou metabolismo de primeira passagem (Oga et al., 2008).

1.1.2.2. Biotransformação

As enzimas de biotransformação são amplamente distribuídas por todo o organismo, porém encontram-se em maior concentração no tecido hepático. (Oga et al., 2008). O fígado quando removido e submetido a homogeneizações seguidas de centrifugações sucessivas a velocidades crescentes, fornece diversas frações das células hepáticas.

Nessas céluas ocorrem as reações de biotransformação de xenobióticos frequentemente classificadas como microssomais ou citosólicas. As enzimas microssomais catalisam a maioria das reações de Fase 1, enquanto as enzimas citosólicas são responsáveis principalmente por biotransformações de Fase 2 (Oga et al., 2008).

A grande parte do paracetamol ingerido é diretamente conjugado sofrendo reação de detoxificação de Fase 2, e posterior sulfatação e glicuronidação para então posteriormente ser excretado. A enzima uridinoglicuroniltrasnferase (UGT) é um conjunto de isoenzimas que reconhecem grupos funcionais específicos e utilizam um substrato em comum, o ácido uridínico 5-difosfato-glicurônico (UDP-glicurônico) em reações de conjugação. Elas são divididas em duas famílias (UGT1 e UGT2) e três subfamílias (UGT1A, UGT2A, UGT2B).

As isoformas UGT 2B15, 1A1, 1A6 e 1A9 estão envolvidas na metabolização do paracetamol. A reação de glicuronidação, por elas realizada, transfere o ácido glicurônico proveninete do ácido UDP-glicurônico para o APAP. É formado assim um conjugado de APAP com ácido glicurônico para então ser excretado (Mutlib et al. 2006).

Nas reações de sulfatação, a superfamília das sulfotransferases (SULT) catalisam a conjugação do sulfato com substâncias endógenas e exógenas. A isoforma SULT 1A1 é uma fenol sulfotransferase que, preferencialmente, catalisa a sulfatação de pequenos fenóis planos assim como o APAP. Após a conjugação com o sulfato, forma-se um complexo no qual o APAP é excretado (Glatt & Meinl 2004).

O sistema microssomal hepático p450 é composto por uma série de enzimas monooxigenases que oxidam compostos endógenos e exógenos. A parcela de APAP metabolizada por esse sistema é convertida em N-acetil-p-benzoquinona-imina (NAPQI). Este, por sua vez, pode se ligar as proteínas celulares causando danos as mesmas. Para proteger a estrutura celular, o NAPQI é neutralizado pela Glutationa S-transferase (GST) através da conjugação com o tri peptídeo glutationa, podendo assim ser eliminado como mostra a Figura 2. (Gonzalez 2005).

A partir de estudos realizados com microssomas hepáticos humanos, tem sido demonstrado que as enzimas do citocromo p450 são as responsáveis pela produção de metabólitos reativos que se ligam as proteínas microssomais. A ativação metabólica pode envolver as isoformas CYP1A1, 1A2, 2E1, 3A1 e 3A2. Aproximadamente 70% das ligações covalentes são com os resíduos de cisteína proveninentes das proteínas hepáticas.(Kim et al. 2001).

Nas ingestões tóxicas de APAP a enzima CYP2E1 foi considerada como sendo a mais eficiente na bioativação do NAPQI. Na formação de 3-hidróxi-paracetamol (3-OH PAR), um metabólito intermediário na detoxificação do APAP, a proporção relativa de NAPQI para formação de 3-OH PAR-foi de aproximadamente 6 para 1. Em seguida a isoforma CYP2A6 apresentou uma proporção de formação de NAPQI numa proporção de 3 para 1 (Zamin et al. 2002). Nos seres humanos, a contribuição relativa de cada uma das isoformas envolvidas varia dependentemente do uso de drogas farmacêuticas ou outros indutores enzimáticos (Kalsi et al. 2011).

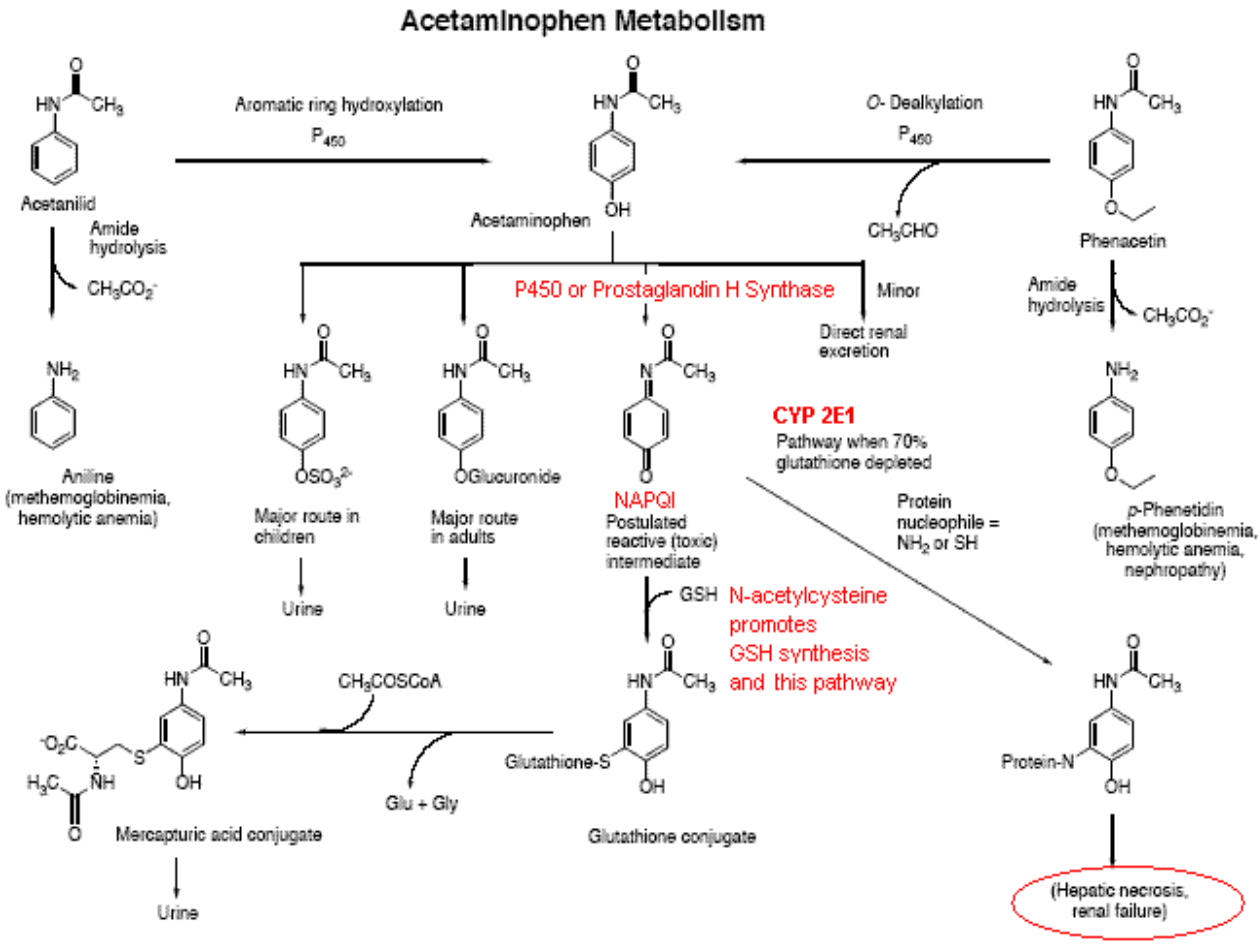

Fig2. Metabolismo do APAP

1.1.2.3. Distribuição

Após a absorção intestinal, o APAP é transportado pelo sangue ligado às proteínas plasmáticas. Ao passar pela maioria dos tecidos ele pode se ligar as células causando danos principalmente no tecido hepático e renal, onde seus danos são mais pronunciados nas intoxicações agudas. Em seguida o mesmo é distribuído nos variados líquidos corporais de forma relativamente uniforme. Isso permite ao APAP a capacidade de atravessar a barreira hematoencefálica, bem como a barreira placentária, onde pode causar danos aos hepatócitos fetais (Carolina et al. 2015).

1.1.2.4. Excreção

Em doses terapêuticas, a maior parte do paracetamol sofre glicuronização ou sulfatação e, assim, pode ser eliminado através de excreção renal ou biliar, conjugado com ácido glicurônico ou sulfato. Apenas uma pequena porção excedente é metabolizada pelo sistema microssomal p450 e produz NAPQI, que é rapidamente conjugada com glutationa reduzida para posterior excreção. A droga pode ser reabsorvida na urina no primeiro dia de ingestão, principalmente depois de conjugada com ácido glicurônico, ácido sulfúrico ou cisteina (Chen et al. 1998);

A excreção de ácido mercaptúrico também pode ocorrer e é classificada como dose dependente. Ela se mostrou crescente nos experimentos em animais depois de administradas sucessivas doses hepatotóxicas. (Kis et al. 2005).

Após uma overdose de APAP ocorre a formação do metabólito reativo NAPQI. A glutationa por sua vez é depletada ao tentar neutralizar o metabólito citado. Quando a reserva de glutationa é depletada o NAPQI passa a danificar as células hepáticas. Normalmente nas intoxicações a taxa de ressíntese de glutatationa reduzida não é suficiente, necessitando assim de administração da mesma. (Kis et al., 2005).

Em humanos, cerca de 30 a 55% do APAP administrado é excretado na urina como conjugado de PAR sulfato (PAR-Sulp) e conjugado de PAR glicuronídeo (PAR-Gluc), respectivamente. Os conjugados de PAR cisteína (PAR-Cys) e conjugados de PAR n-acetil-cisteína (PAR-NAC) contribuem para 4% da dose. (Bessems & Vermeulen 2001).

Os rins e intestino também estão envolvidos no processamento dos metabólitos APAP. Ambos os órgãos contêm a enzima Gama Glutamil Transpeptidase (GGT) e Dipeptidase. Estas enzimas

atuam na quebra dos conjugados de PAR. Entre eles: PAR copnjugado de glutationa (PAR-SG), PAR conjugado de cisteinil glicina (PAR-CG) e PAR conjugado de cisteína (PAR-Cis). Os rins também desempenham um papel importante no processamento dos metabólitos sulfurados doPAR, seja por filtração glomerular ou por biotransformação com subsequente excreção . (Farrell et al. 1997).

1.1.3.Toxicodinâmica e Efeitos tóxicos do paracetamol

1.1.3.1. Dano Hepático

Doses maiores que 140mg/kg ou 7,5g de APAP são consideradas tóxicas podendo desencadear necrose centro-lobular severa ou fatal. Excedida a dose terapêutica, as vias da sulfatação e da glicuronidação ficam saturadas. Isso resulta em um aumento da produção de NAPQI por meio das enzimas do sistema P450. Esse metabólito gera um aumento do consumo de glutationa reduzida. (Bergman et al.,1996). Ainda no caso de uma overdose o APAP se conjuga covalentemente as moléculas de DNA ou outras proteínas causando citotoxicidade em várias linhagens celulares. (Wlodek et al.,1997).

Outros mecanismos de lesão ocorrem com alterações mitocondriais e consequente diminuição da produção de energia. Várias proteínas envolvidas no equilíbrio iônico podem ser afetadas prejudicando assim o funcionamento fisiológico das células. A perda deste funcionamento correto por parte das mitocôndrias ou do núcleo leva ao aumento do Ca^{2+} citosólico e mitocondrial. Este aumento por sua vez pode induzir a ativação de proteases e endonucleases e por consequência, lesões na molécula de DNA. O resultado disso pode ser a perda da atividade ou função de muitas proteínas, com eventual morte ou lise celular (James et al.,2003).

O estresse oxidativo também apresenta relação com a hepatotoxicidade. Através do aumento na formação do metabólito NAPQI ocorre uma depleção nos níveis celulares de Glutationa reduzida. Isso faz com que a enzima glutationa peroxidase passe a ter sua atividade também diminuída, resultando no acúmulo de peróxidos. O estresse oxidativo também pode ocorrer em consequência de

uma via de co-produção do radical superóxido e peróxido de hidrogênio, estes decorrentes do ciclo redox entre o radical semiquinona do APAP e o NAPQI (James et al., 2003).

Experimentos em espécies animais suscetíveis ao paracetamol têm sido realizados com o objetivo de elucidar os mecanismos desconhecidos da hepatotoxicidade. Foram encontrados diversos graus de necrose hepática quando administrado o PAR em animais pré- tratados com indutores de enzimas microssomais. Por outro lado, também foi observado que o dano foi reduzido em animais que receberam inibidores dessas enzimas. Similarmente, em humanos expostos a drogas indutoras enzimáticas como fenobarbital ou etanol estão mais suscetíveis a hepatotoxicidade do paracetamol. Pelo contrário, tem sido descrita resistência a esses efeitos nos pacientes submetidos ao uso de cimetidina, um inibidor do sistema de enzimas microssomais p450 (Grattagliano et al., 2009).

Estudos citoquímicos mais recentes utilizando anticorpos anti-paracetamol têm mostrado que a ligação covalente dos metabólitos do paracetamol ocorre nas regiões danificadas centrolobulares do fígado humano após a sobredosagem (Greene et al., 2006). Além dos danos hepáticos podem ocorrer necrose tubular renal e coma hipoglicêmico.

1.2 Artemísia Absínthium

Em busca da prevenção da hepatotoxicidade induzida por APAP, vários estudos são realizados procurando o potencial antioxidante por plantas medicinais (Craciunescu et al 2012).

A Artemísia Absinthium é uma espécie originária da Europa, cultivada no Brasil por suas propriedades medicinais. É uma planta herbácea de caule ramificado, ereto, áspero de cor verde-prateada, com até um metro de altura. Apresenta em suas folhas e flores, além do olho essencial Tujona, vários compostos orgânicos de interesse medicinal.

E dentre as plantas medicinais, a Artemísia Absinthium, popularmente conhecida pelo seu sabor amargo, e comumente chamada Losna aparece como uma possível terapia em questão de hepatotoxicidade induzida por xenobióticos.

1.2.1 Artemísia Absínthium na intoxicação por APAP

Assim como na maioria das plantas, na Artemísia Absinthium estão presentes compostos como mono e sesquiterpenos, ácidos fenólicos e lactonas. Ainda entre as principais substâncias estudadas nessas plantas os polifenóis e os flavonoides merecem destaque. Estes compostos podem minimizar o estresse oxidativo associado a diversas condições patológicas (Grassi et al 2010).

A Artemisia Absinthium (AA) como uma planta medicinal já mostrou ter efeitos benéficos na contra a hepatotoxicidade induzida por tetracloreto de carbono em camundongos (Saxena&Shukla 2012). Estes efeitos podem estar relacionados pelo fato de AA possuir uma quantidade significativa de flavonoides e polifenóis, como: rutina, quercetina e kampferol (Fakurazi et al. 2012), e também por apresentar uma baixa toxicidade até 100µg/µL em modelos animais experimentais (Amat et al., 2010).

Ela é uma das setenta e uma plantas selecionadas pelo ministério da saúde como de interesse ao Sistema Único de Saúde, onde é indicada contra problemas de estômago, fígado, rins e verminoses. Além disso, a AA tem propriedades antimicrobianas contra microorganismos gram-negativos em crescimento (Moslemi et al., 2012) além de outros parasitas de interesse clínico (Tariq et al., 2009).

Todos estes dados e resultados da literatura científica nos levam a necessidade de estudos mais aprofundados na investigação das possíveis propriedades terapêuticas ou farmacológicas da AA. Modelos agudos, crônicos ou ainda sub crônicos intoxicados com diferentes concentrações de APAP e tratados com AA por gavagem são uma boa forma de estudar AA na hepatotoxicidade induzida.

No entanto, os efeitos de diferentes extratos de AA contra a hepatotoxicidade induzida por APAP, ainda não foram investigados nos modelos descritos acima. As investigações de tais extratos na prevenção da hepatotoxicidade induzida ainda não estão totalmente descritas pela literatura e fazem parte dos objetivos centrais deste trabalho.

OBJETIVOS

2.1 Objetivos Gerais

Avaliar os efeitos bioquímicos do extrato aquoso de folhas de *Artemísia Absinthium* em um modelo de Hepatotoxicidade induzida por paracetamol em camundongos.

2.2 Objetivos específicos

Identificar os possíveis mecanismos de ação antioxidante deste extrato em ensaios bioquímicos *in vitro* e *ex vivo.*

Identificar ***in vitro:***

- A quelação do radical DPPH (Wu et al., 2006).
- A peroxidação lipídica (formação de espécies reativas do ácido tiobarbitúrico - TBARS) induzidas por ferro em tecidos biológicos de camundongos não tratados (Okawa et al. ,1979).

Identificar ***ex vivo:***

A peroxidação lipídica via espécies reativas do ácido tiobarbitúrico (TBARS) de acordo com Okawa et al. (1979).

A viabilidade celular analisada com base na redução de 3- (4,5-dimethylthiazol- 2-il) brometo de 2,5-difeniltetrazólio (MTT) (0,5 mg / mL) de acordo com Babot et al. (2005).

Os níveis de grupos sulfidrila não proteicos (NPSH) por meio de reação com DTNB, segundo Ellman et al. (1959).

A atividade da enzima antioxidante catalase (CAT) analisada com base na degradação do peróxido de hidrogênio (H2O2) de acordo com Aebi et al. (1984).

A atividade da enzima superóxido dismutase (SOD) determinada através da degradação da adrenalina e da sua inibição, de acordo com Misra e Fridovich et al. (1972).

Os níveis séricos das enzimas aspartato aminotransferase (AST) e alanina amino transferase (ALT) determinados utilizando kits comerciais (Labtest, Minas Gerais / Brasil).

O teor de proteínas determinadas nas amostras de tecidos em todos os animais de acordo Bradford (1976) utilizando albumina de soro bovino (BSA) para a construção de uma curva padrão.

1. DESENVOLVIMENTO

O desenvolvimento que faz parte desta dissertação está apresentado sob a forma de artigo científico. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas, encontram-se no próprio artigo. O artigo encontra-se na formatação de publicação da revista científica The Indian Journal of Medical Research.

Artemisia absinthium minimize acetaminophen- induced toxicity in mice

Fernando W. Franco[1], Órlis M. Lenz[1], Guilherme P. Amaral[1], Silvio T. Stefanelo [1], Diane D. Hartmann[1], Rodrigo P. Martins[1], Aline A. Boligon[2], Margareth L. Athayde[2], Nilda B. V. Barbosa[1], Félix A.A. Soares[1], Gustavo O. Puntel[3*]

[1] Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Santa Maria, UFSM - Santa Maria, RS, Brasil;

[2] Departamento de Farmácia Industrial, Universidade Federal de Santa Maria, UFSM - Santa Maria, RS, Brasil;

[3] Departamento de Morfologia, Universidade Federal de Santa Maria, UFSM - Santa Maria, RS, Brasil;

*CORRESPONDING AUTHOR:

Gustavo Orione Puntel – Departamento de Morfologia - Universidade Federal de Santa Maria, UFSM Campus Camobi 97105-900, Santa Maria, RS, Brasil;

Phone: +55-XX-55-3220-8736

Fax: +55-XX-55-3220-8978

E-mail:gustavopuntel@yahoo.com.br

ABSTRACT

Background and Objectives: Acetaminophen is a popular analgesic associated with toxic ingestions. There are many studies about prevention of APAP induced hepatotoxicity including the medicinal plants. *Artemisia absinthium* (AA) is one of them, but its extract against the hepatotoxicity induced by APAP were not yet investigated. **Methods:** This study aimed to investigate the effects of AA's aqueous extract against the APAP's intoxication in mice. **Results:** Our results show that AA present high poliphenol's concentrations and flavonoids. *In vitro* tests, AA decreased the basal and pro-oxidants induced lipid peroxidation in mice homogenated tissues (10 to 25µg). Also, DPPH radical's (2,2-diphenyl-1-picrylhydrazyl) scavenger activity was reduced (100µg) ($p \leq 0.05$). *Ex vivo*, AA reduced the lipid peroxidation, induced by iron reduced, in mice liver (from 10 to 100µg) and kidneys (at 100µg) ($p \leq 0.05$). The intoxication with APAP determined a significant increase in lipid peroxidation and also a decrease in non-protein thiol levels and of the enzymes superoxide dismutase e catalase activities ($p \leq 0.05$). Moreover, the APAP depicted an increase of the transaminase enzymes activities in serum, and also decreased the cell viability in liver and brain's mice ($p \leq 0.05$). **Conclusions:** Our results show that the aqueous extract of AA may have hepatoprotective properties against toxicity induced by APAP. In general, we suggest that the hepatoprotective effects of AA against an intoxication with APAP were related to its antioxidant potential *in vitro* and *ex vivo.* Finally, further studies are needed to highlight the mechanism of AA aqueous extract action, especially its effects in the mitochondrial dysfunction prevention.

Keywords: hepatotoxicity, acetaminophen, antioxidant, *Artemisia absinthium*.

INTRODUCTION

Acetaminophen (N-acetyl-p-amino phenol) (APAP) is a popularly used analgesic and often is described as a drug associated with toxic ingestions (Shaw, 2013). Despite its use in health treatments by Von Mering since 1893, the APAP's popularity dates from 1949 (Goodman et al., 2005). Among its uses in medicine, APAP is extensively employed to treat mild to moderate pain, related to several situations such as arthritis, headache, myalgia, dysmenorrhea, dental disorders and fever (Saska et al. 2009)(Krenzelok & Royal 2012).

A large part of the APAP is conjugated with glucuronic acid, glutathione and sulphate suffering detoxification reaction Stage 2 and then excreted (Schiødt et al. 1997)(Krishnan et al. 2013). The rest of APAP is mainly metabolized by hepatic cytochrome P450 in N-acetyl-p-benzoquinone imine (NAPQI), which produce reactive oxygen species (ROS) (Moyer et al. 2011, Carvalho et al. 2013). Further, the NAPQI is generally neutralized by glutathione S-transferase (GST) through conjugation with reduced glutathione (GSH) to prevent cell damage. (Dai et al. 2005).

The overdose of APAP can overload the metabolizing pathways causing the increased NAPQI formation and depletion of cellular GSH (Bunchorntavakul et al., 2013). Excessive amounts of NAPQI also can covalently bind to essential cellular protein leading to hepatocyte death and liver injury possible due to mitochondrial dysfunction (Carvalho et al. 2013). Mitochondrial dysfunction determines an increased formation of ROS, which contributes to the decrease in GSH, the major endogenous antioxidant defense in hepatocytes, leaving these cells vulnerable to oxidative damage (Jaeschke et al. 2006).

Seeking prevention of APAP's induced hepatotoxicity, several studies are performed looking for the potential antioxidant by medicinal plants (Craciunescu et al. 2012). This compounds can minimize the oxidative stress associated with pathological conditions (Grassi et al. 2010).

In line with this, the *Artemisia absinthium* (AA) as a medicinal plant has already shown to have hepatoprotective effects against the toxicity determined by carbon tetrachloride in mice (Saxena & Shukla 2012). In fact, AA has significant amount of flavonoids, such as rutin, quercetin and kaempferol (Fakurazi et al. 2012), and also a low toxicity in experimental animal models (Amat et al. 2010). However, the effects of an aqueous extract of AA against the hepatotoxicity induced by APAP were not yet investigated.

Therefore, this study aimed to investigate the antioxidant effects of AA's aqueous extract in *in vitro* and *ex vivo,* in experimental mice submitted or not to APAP's intoxication. In this context, we investigated the oxidative damage determined by acute APAP's intoxication and also the protective effects of AA's aqueous extract.

MATERIAL AND METHODS:

Chemical reagents

For high performance liquid chromatography (HPLC-DAD) analysis de reagents methanol, acetic acid, gallic acid, chlorogenic acid, ferulic acid and caffeic acid were purchased from Merck (Darmstadt, Germany). The reagents quercetin, quercitrin, isoquercitrin, rutin, kaempferol, catechin, epicatechin, acetaminophen (APAP), tiobarbituric acid (TBA), malondialdehyde (MDA), reduced glutathione (GSH), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) 20mM, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) anddichlorofluorescindiacetate (DCF) were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA). Alanine aminotransferase (ALT) e Aspartate aminotransferase (AST) diagnostic kits were purchased from LabtestDiagnóstica (Minas Gerais/Brazil). All the other reagents were obtained form local suppliers.

Artemisia absinthium (AA) extract preparation

The AA was prepared with naturally dried leaves. AA leaves were collected from the campus of the Federal University of Santa Maria -RS, Brazil, in April of 2014. The botanical identification of the samples was confirmed and a voucher specimen (number ICN 9511; *Artemisia Absinthium*) was deposited at the ICN Herbarium of the Federal University of Santa Maria (UFSM). The leaves were dried at room temperature for one month.

First, the leaves were separated from the stems and then dried in the shade. Subsequently, were weighed and placed in an extraction funnel containing hot water, where the extraction of AA's aqueous fraction was made. Then the obtained solution was filtered and progressively evaporated in a thermostated bath at 50 °C, until AA powder had obtained.

In vitro assays

HPLC analysis

HPLC-DAD of the AA's aqueous extract was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software.

DPPH$^{\bullet}$ scavenging activity

The AA tea scavenger activity against the 2,2-diphenyl-1-picrylhydrazyl(DPPH$^{\bullet}$) radical was performed according to (Wu et al. 2006).

AA aqueous extract effects against basal and induced lipid peroxidation

The analysis of AA aqueous extract effects against basal and pro oxidants induced lipid peroxidation was performed using different mice tissues. Mice were killed by decapitation and tissues were removed, quickly homogenized in NaCl (150mM) and kept in ice. After the homogenization, samples were centrifuged (4,000 x *g* at 4ºC for 10 min) to yield a low speed supernatant fraction (S1) that was incubated in a reaction medium containing Tris-HCl (1:10; 10 mM; pH 7.4), different concentrations of AA aqueous extract, and with or without reduce iron (Fe^{2+} 10µM) or sodium nitroprusside (SNP 5µM). This mixture was incubated at 37ºC for 60 min and after used in the lipid peroxidation assay (described below).

STUDY DESIGN:

***Ex vivo* assays**

Experimental animals

Swiss, adult, male and albino mice were obtained from own breeding colony and maintained in an air-conditioned room (20–25 ◦C) under a 12 h light/dark cycle, and with water and food *ad libitum*. All the animals were maintained and used in accordance with guidelines of the Committee on Care and Use of Experimental Animal Resources (process number 115/2013) of the Federal University of Santa Maria, Brazil.

Mice were divided in five different groups (n=5) that received or not AA's aqueous extract (1-100 µg/µL) via gavage (10ml/Kg p.o.) for three days. The mice that not received AA aqueous extract were treated with distilled water that was used as vehicle. After 72 hours of the administration for AA aqueous extract or of the vehicle administration, the mice were euthanized and its liver, kidneys and brain tissues were removed to perform biochemical analysis. This first step was performed in

order to analyze the AA aqueous extract toxicity per se and also to define the AA's tea concentration used in an APAP's intoxication model.

Mice were divided in four experimental groups (n=5) as follows:

1. Control (n=5): Mice that received distilled water via gavage (10mL/Kg) for four weeks and further injected with saline solution that was used as vehicle (150mM; pH 7,4 i.p);

2. APAP (n=5): Mice that received distilled water via gavage (10mL/Kg) for four weeks and further injected with a single dose of APAP (600mg/kg, i.p.) freshly dissolved in saline solution (0.150M; pH 7.4);

3. AA (n=5): Mice that received AA aqueous extract via gavage (10 µg/µL p.o.) (10mL/Kg) daily for four weeks and further injected with saline solution (0.150M; pH 7.4 i.p.);

4. APAP+AA (n=5): Mice that received AA aqueous extract via gavage (10 µg/µL p.o.) (10mL/Kg) daily for four weeks and further injected with APAP (600 mg/kg, i.p.);

Body's mice weight were measured before the APAP or vehicle injection. All the animals were fasted for a period of six hours. After 6 hours of APAP or vehicle administration the mice were euthanized and whole blood, liver, kidneys and brain tissues were removed to perform biochemical analysis.

Tissue preparation

The whole blood was collected by cardiac puncture in non heparinized tubes and centrifuged (2.000 X g for 10 min) in order to separate the cellular blood fraction and the serum. The serum was used for biochemical analysis. Liver, kidney, brain and tissue's mice were quickly removed and placed on ice until homogenization in cold Tris-HCl buffer solution (1:10; 10mM pH 7.4). The homogenates were centrifuged at 2000×g at 4ºC for 10 min to yield a low-speed supernatant fraction (S1) that was used for biochemical analysis.

Biochemical analysis

The lipid peroxidation was accessed via thiobarbituric acid reactive species (TBARS) formation according to Okawa et al. (1979).

The cell viability was analyzed based on reduction of 3-(4,5-dimethylthiazol- 2-yl) 2,5-diphenyltetrazolium bromide (MTT) (0.5 mg/mL) according to Babot et al. (2005).

The non-protein sulphydryl groups (NPSH) levels were determined via DTNB reduction according to (Ellman 1959).

The antioxidant enzyme catalase (CAT) activity was analyzed based on hydrogen peroxide (H2O2) degradation according to Aebi et al. (1984).

The activity of superoxide dismutase (SOD) enzyme was determined via epinephrine degradation's and its inhibition according to Misra and Fridovich et al. (1972).

Serum enzymes of aspartate aminotransferase (AST) and activity of alanine aminotransferase (ALT) were determined using commercial kits (Labtest, Minas Gerais/Brazil).

Protein content was determined in all mice tissue S1 samples according Bradford (1976) using bovine serum albumin (BSA) to construct a standard curve.

Statistical analysis

Data were analyzed by one-way ANOVA followed by Tukey's test, using the software GraphPad Prism 5.0 for windows. Differences between groups were considered significant when $p \leq 0.05$.

RESULTS

AA aqueous extract effects *in vitro*

The HPLC profile of AA aqueous extract, in Figure 3, reveled the following main compounds: gallic acid (retention time-tR 11.39 min, peak 1), catechin (tR = 14.97 min, peak 2), chlorogenic acid (tR = 18.73 min, peak 3), caffeic acid (tR = 21.08 min, peak 4), ferulic acid (tR = 24.11 min, peak 5); epicatechin (tR = 29.37 min, peak 6), rutin (tR = 35.16 min, peak 7), quercitrin (tR = 39.85 min, peak 8), isoquercitrin (tR = 42.07 min, peak 9), quercetin (tR = 44.81 min, peak 10) and kaempferol (tR = 49.73 min, peak 11). The main concentrated polyphenols founded was ferulic acid followed by caffeic acid and gallic acid. In flavonoid composition the main concentrated in AA aqueous extract was quercetin followed by kaempferol. These and other phenolic and flavonoid compounds founded and their concentrations shows in Table 1.The AA aqueous extract showed a significant $DPPH^{\bullet}$ scavenger activity only at 100µg ($p \leq 0.05$) (Figure 4).

Both pro-oxidants used induced a significant increase in TBARS levels in all of the tissue's mice analyzed ($p \leq 0.05$). The AA aqueous extract decreased the induced TBARS levels to control values at 10µg in liver and brain, and at 25µg in kidneys. Moreover the basal TBARS levels (none induced by pro-oxidants) were also significantly decreased by AA at 10µg in brain, and at 25µg in liver ($p \leq 0.05$). None effect of AA was observed in basal TBARS levels of kidneys (Table 2).

AA aqueous extract effects *per se* on experimental animals

The *ex vivo* effects of AA aqueous extract *per se* on mice liver were show in Table 3. The increased TBARS values induced by Fe^{2+} (10µM) were counteracted by AA treatment from 10 to 100µg concentrations ($p \leq 0.05$). Moreover, basal lipid peroxidation and the NPSH, DCFA and MTT reduction levels were not changed by AA aqueous extract treatment in liver.

In kidneys, theFe^{2+} induced lipid peroxidation was significantly decreased by AA treatment only at 100µg concentration ($p \leq 0.05$) (Table 4). The kidneys NPSH and DCFA levels were also decreased by AA aqueous extract at 100µg and from 50 to 100µg, respectively ($p \leq 0.05$) (Table 4). The MTT reduction levels were not changed by AA aqueous extract treatment in kidneys.

In table 5 were shown the *ex vivo* effects of AA aqueous extract treatment in brain. In this tissue the AA treatment was unable to decrease the Fe^{2+} induced lipid peroxidation. Furthermore, the basal lipid peroxidation and the NPSH, DCFA and MTT reduction levels were not changed by AA aqueous extract treatment in brain.

AA aqueous extract effects on APAP intoxication

In Figure 5 was showed the effects of AA aqueous extract on mice serum ALT (5A) and AST (5B) enzymes activities. Is possible to observe that APAP induced a significant increase both in ALT and AST activities and the pre treatment with AA significantly reduced this increase ($p \leq 0.05$).

APAP's intoxication increased significantly the lipid peroxidation in mice liver (Fig 6A), kidneys (Fig 6B) and brain too (Fig 6C) ($p \leq 0.05$). The AA's pre treatment reduced TBARS levels only in liver (Fig 6A) and in brain (Fig 6C) ($p \leq 0.05$).

The cellular viability measured via MTT reduction test was significantly reduced by APAP in m ice liver (Fig 7A) and brain (Fig 7C), but not in kidneys (Fig 7B) ($p \leq 0.05$). In liver and in brain the AA's aqueous extract restored the MTT reduction activity to control values (Figures 7A and 7C, respectively).

The NPS levels were significantly reduced by APAP and the AA pre treatment counteracted these NPSH depletion in all the mice tissue tested (Figure 8) ($p \leq 0.05$).

The CAT and SOD enzyme activities were significantly reduced in all the mice tissue tested (Figure 9 and Figure 10, respectively) and the AA pre treatment was not effective to restore their activities to control values ($p \leq 0.05$)

Table 1 – Phenolics and flavonoids composition of *Artemisia absinthium*.

Compounds	*Aqueous extract*		*LOD*	*LOQ*
	mg/g	%	µg/mL	µg/mL
Gallic	36.17 ± 0.03a	3.61	0.018	0.059
Catechin	5.03 ± 0.01b	0.50	0.023	0.075
Chlorogenic acid	12.09 ± 0.01c	1.20	0.007	0.023
Caffeic acid	41.73 ± 0.02d	4.17	0.034	0.112
Ferulic acid	42.95 ± 0.03d	4.29	0.011	0.034
Epicatechin	15.20 ± 0.01e	1.52	0.015	0.049
Rutin	7.46 ± 0.02f	0.74	0.029	0.095
Quercitrin	20.07 ± 0.01g	2.00	0.042	0.138
Isoquercitrin	18.92 ± 0.03h	1.89	0.031	0.102
Quercetin	30.11± 0.02i	3.01	0.008	0.029
Kaempferol	29.67 ± 0.01i	2.96	0.021	0.069

Results are expressed as mean ± S.E.M. of three determinations. LOD limit of detection (µg/ml), LOQ limit of quantification (µg/ml). Averages followed by different letters differ by Tukey test at $p \leq 0.05$.

Table 2: In vitro effects of AA aqueous extract against basal and induced TBARS levels increase in mice:

AA aqueous extract µg/µL	Liver			Kidneys			Brain		
	Basal	Fe^{2+}	SNP	Basal	Fe^{2+}	SNP	Basal	Fe^{2+}	SNP
0	24.09±1.40	33.31±3.44*	33.59±2.28*	7.30±1.63	16.59±2.59*	16.23±2.37*	10.89±1.42	23.09±1.60*	26.79±3.07*
10	15.80±4.59	23.64±0.95#	25.36±1.21#	6.39±2.13	10.88±4.10	12.58±5.02	2.84±0.74#	7.69±1.40#	12.01±4.46#
25	11.12±3.23#	13.74±4.74#	17.13±4.23#	5.80±1.68	8.64±1.45#	9.39±4.42#	1.80±1.16#	2.98±0.56#	6.53±0.44#
50	10.03±2.54#	14.25±4.45#	15.34±4.12#	4.73±1.14	7.76±2.13#	7.40±2.66#	2.09±0.69#	2.26±0.51#	5.38±1.53#
75	7.86±2.15#	6.35±1.77#	6.90±2.07#	5.08±1.20	4.93±1.76#	9.24±3.77#	1.66±1.40#	2.92±0.48#	2.25±0.26#
100	5.85±0.36#	5.50±0.14#	6.53±3.09#	4.27±1.25	4.29±1.58#	8.47±4.5#	1.63±0.90#	2.10±1.57#	2.08±1.53#

Data (mean±S.E.M.) (n=4-6) were expressed in nmol MDA/ mg of protein.

* Shows significant difference in comparison to basal lipid peroxidation of respective tissue without pro-oxidant, $p \leq 0.05$.

Shows significant difference of several AA aqueous extract in comparison to respective tissue and basal or pro-oxidant induced lipid peroxidation, $p \leq 0.05$.

Table 3: *Ex vivo* effects of AA aqueous extract on TBARS (basal and induced), NPSH, DCFA, MTT levels in mice liver.

AA aqueous extract µg/µL	TBARS Basal	TBARS Induced	NPSH	DCFA	MTT
0	0.925±0.013	1.805±0.264*	10.282±0.267	8.505±0.043	99.588±0.238
1	0.891±0.041	1.685±0.248*	10.943±0.148	9.390±0.121	87.627±4.494
10	0.728±0.020	1.504±0.191	9.704±1.060	9.340±0.095	88.364±3.776
50	0.688±0.048	1.321±0.196	8.694±0.009	9.020±0.118	89.212±3.449
100	0.504±0.024	1.301±0.186	8.819±0.276	8.757±0.062	90.442±5.071

Data were expressed as mean±S.E.M. (n=4-6). TBARS (basal or induced by Fe^{2+} 10µM after 1hour of pre-incubation at 37 °C) values were expressed in nmol MDA/ mg of protein. NPSH values were expressed in µmol SH/ mg of protein. DCFDA values were expressed in nmol oxidized-DCFA-DA/ mg of protein. MTT values were expressed in percentage of control (without AA aqueous extract).

* Shows significant difference in comparison to basal lipid peroxidation (TBARS basal without AA aqueous extract), $p \leq 0.05$.

Shows significant difference of several AA concentrations in comparison to respective analysis without AA aqueous extract, $p \leq 0.05$.

Table 4: *Ex vivo* effects of AA aqueous extract on TBARS (basal and induced), NPSH, DCFA, MTT levels in mice kidneys:

AA aqueous extract µg/µL	TBARS Basal	TBARS Induced	NPSH	DCFA	MTT
0	0.775±0.0001	2.050±0.162*	17.243±1.287	10.460±0.514	100.475±0.274
1	0.775±0.0173	1.821±0.218*	17.383±1.270	10.400±0.534	95.385±2.838
10	0.571±0.0179	1.654±0.132*	13.643±0.047	9.563±0.076	100.570±2.973
50	0.627±0.0370	1.524±0.102*	12.662±1.695	8.825±0.170#	101.757±2.565
100	0.412±0.0180	1.309±0.214#	10.054±0.682#	8.715±0.121#	97.945±0.944

Data were expressed as mean±S.E.M. (n=4-6). TBARS (basal or induced by Fe^{2+} 10µM after 1hour of pre-incubation at 37 °C) values were expressed in nmol MDA/ mg of protein. NPSH values were expressed in µmol SH/ mg of protein. DCFDA values were expressed in nmol oxidized-DCFA-DA/ mg of protein. MTT values were expressed in percentage of control (without AA aqueous extract).

* Shows significant difference in comparison to basal lipid peroxidation (TBARS basal without AA aqueous extract), $p \leq 0.05$.

Shows significant difference of several AA concentrations in comparison to respective analysis without AA aqueous extract, $p \leq 0.05$.

Table 5: *Ex vivo* effects of AA aqueous extract on TBARS (basal and induced), NPSH, DCFA, MTT levels in mice brain:

AA aqueous extract µg/µL	TBARS Basal	TBARS Induced	SHNP	DCFA	MTT
0	0.775±0.0006	2.621±0.335*	13.726±0.147	4.910±0.277	97.995±1.158
1	0.776±0.0172	2.698±0.183*	14.363±0.414	5.008±0.065	90.300±4.500
10	0.571±0.0179	2.337±0.149#	13.430±0.083	4.968±0.157	94.270±3.831
50	0.627±0.0368	2.321±0.180*	13.548±0.413	4.323±0.198	89.990±0.294
100	0.412±0.0180	2.361±0.061*	11.763±1.524	4.280±0.130	91.683±1.171

Data were expressed as mean±S.E.M. (n=4-6). TBARS (basal or induced by Fe^{2+} 10µM after 1hour of pre-incubation at 37 °C) values were expressed in nmol MDA/ mg of protein. NPSH values were expressed in µmol SH/ mg of protein. DCFDA values were expressed in nmol oxidized-DCFA-DA/ mg of protein. MTT values were expressed in percentage of control (without AA aqueous extract).

* Shows significant difference in comparison to basal lipid peroxidation (TBARS basal without AA aqueous extract), $p \leq 0.05$.

Shows significant difference of several AA concentrations in comparison to respective analysis without AA aqueous extract, $p \leq 0.05$.

FIG 3

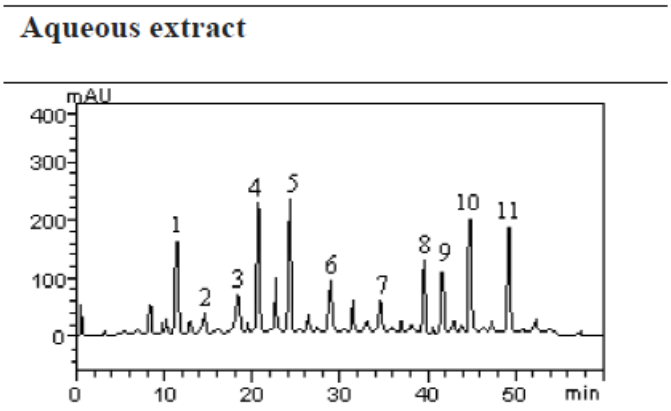

Figure 3: High performance liquid chromatography phenolics and flavonoids profile of aqueous extract of AA. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ferulic acid (peak 5), epicatechin (peak 6), rutin (peak 7), quercitrin (peak 8), isoquercitrin (peak 9), quercetin (peak 10) and kaempferol (peak 11).

Figure 4:

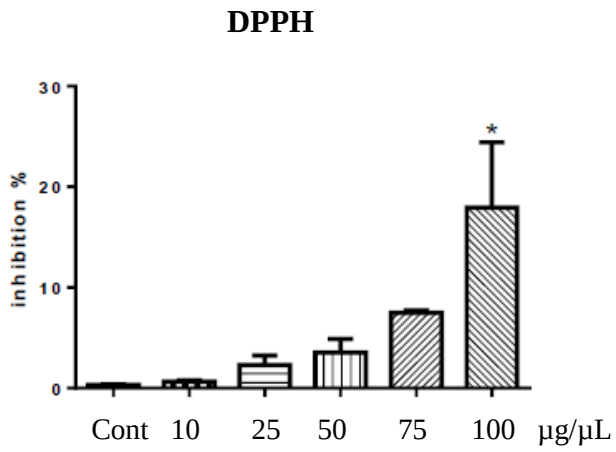

Figure 4: DPPH scavenger activity of AA aqueous extract. Data are expressed as mean ± S.E.M. (n = 4-6) and significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant differences were indicated by * $p < 0.05$; where * denotes difference to control group.

Figure 5:

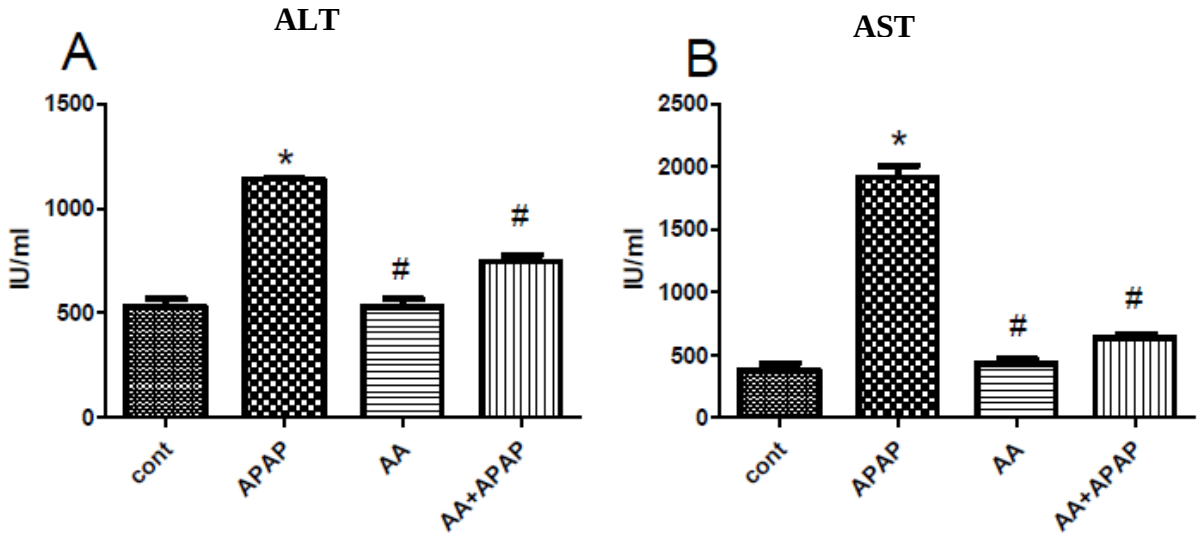

Figure 5: Effect of APAP and AA in serum ALT(A) and AST(B) enzymes of mice. Dates were expressed as mean ± SEM (n = 4-6) and significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant differences were indicated by *or# $p < 0.05$; where * denotes difference to control group and # differences to APAP group.

Figure 6:

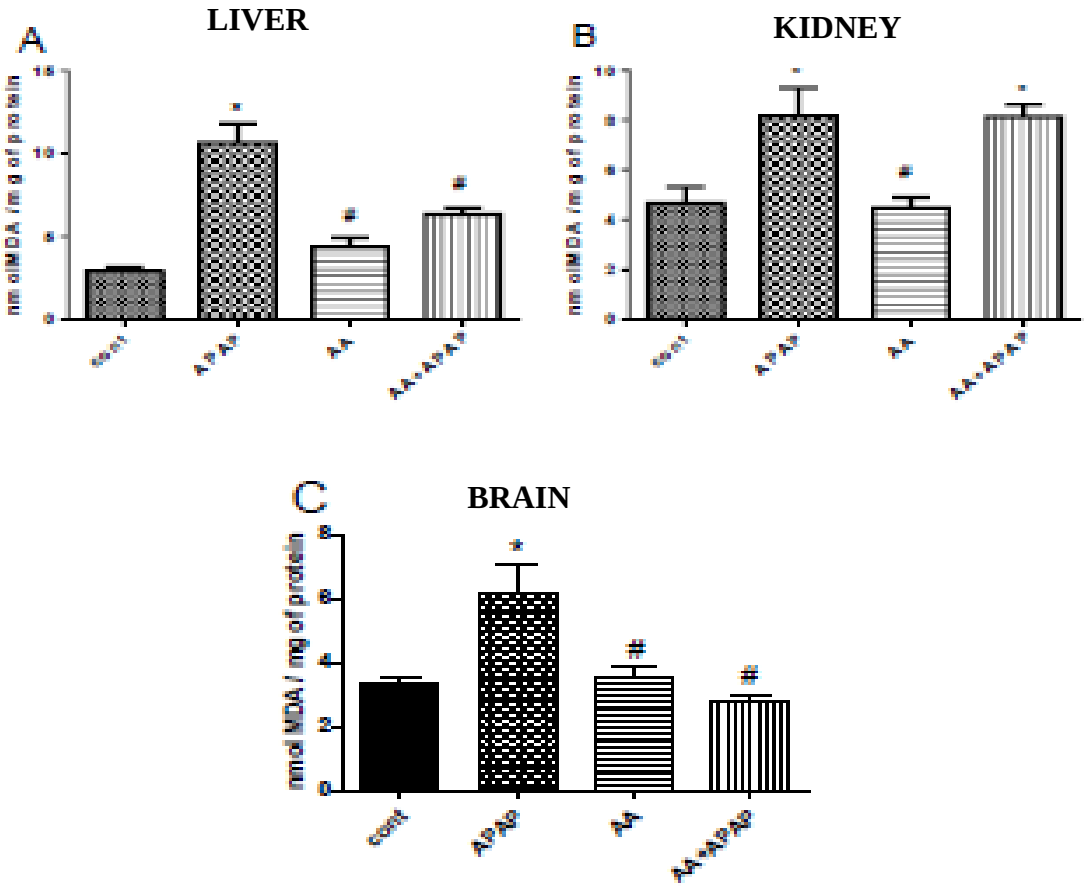

Figure 6: Effect of APAP and AA in TBARS levels in mice tissues: (A) liver; (B) kidneys and (C) brain. Dates were expressed as mean ± SEM (n = 4-6) and significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant differences were indicated by *or# p <0.05; where * denotes difference to control group and # differences to APAP group.

Figure 7:

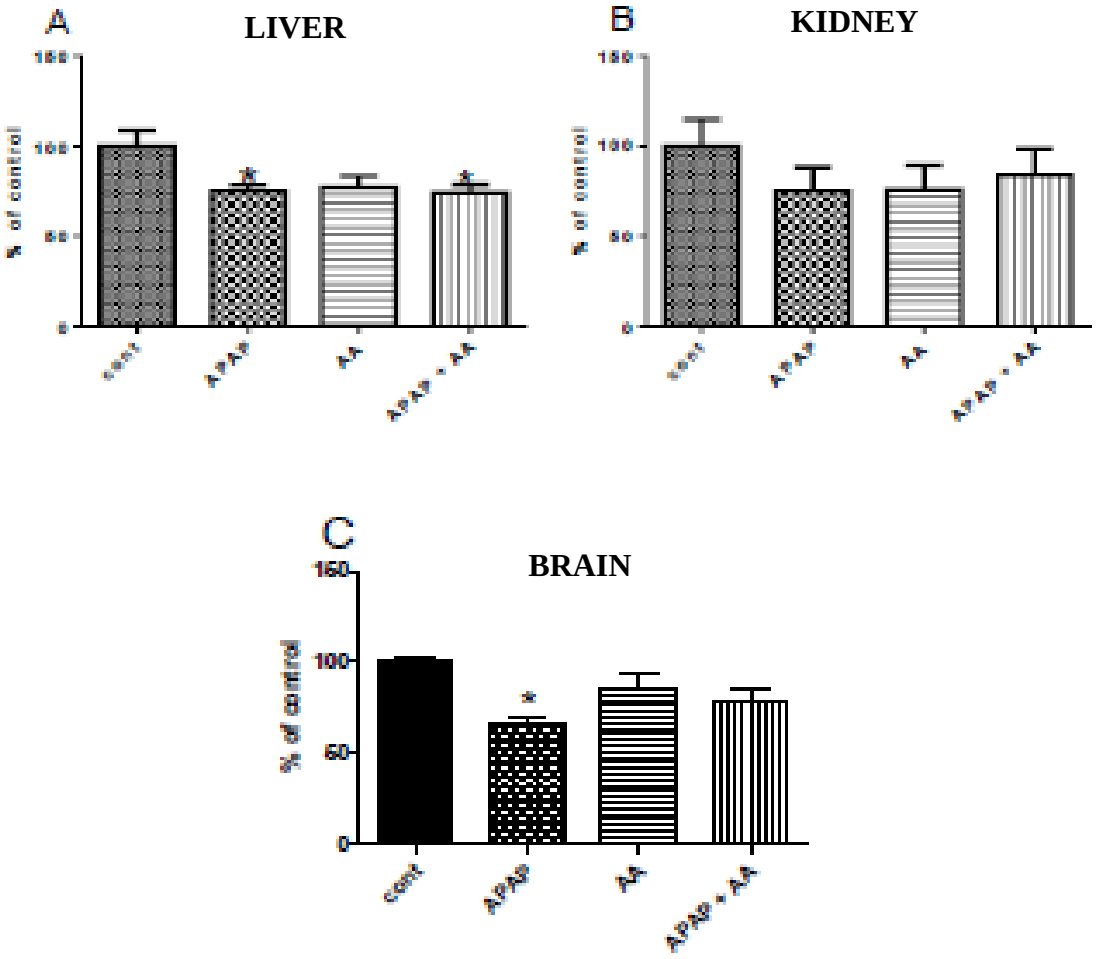

Figure 7: Effect of APAP and AA in MTT levels in mice tissues: (A) liver; (B) kidneys and (C) brain. Dates were expressed as mean ± SEM (n = 4-6) and significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant differences were indicated by *or# $p < 0.05$; where * denotes difference to control group and # differences to APAP group.

Figure 8:

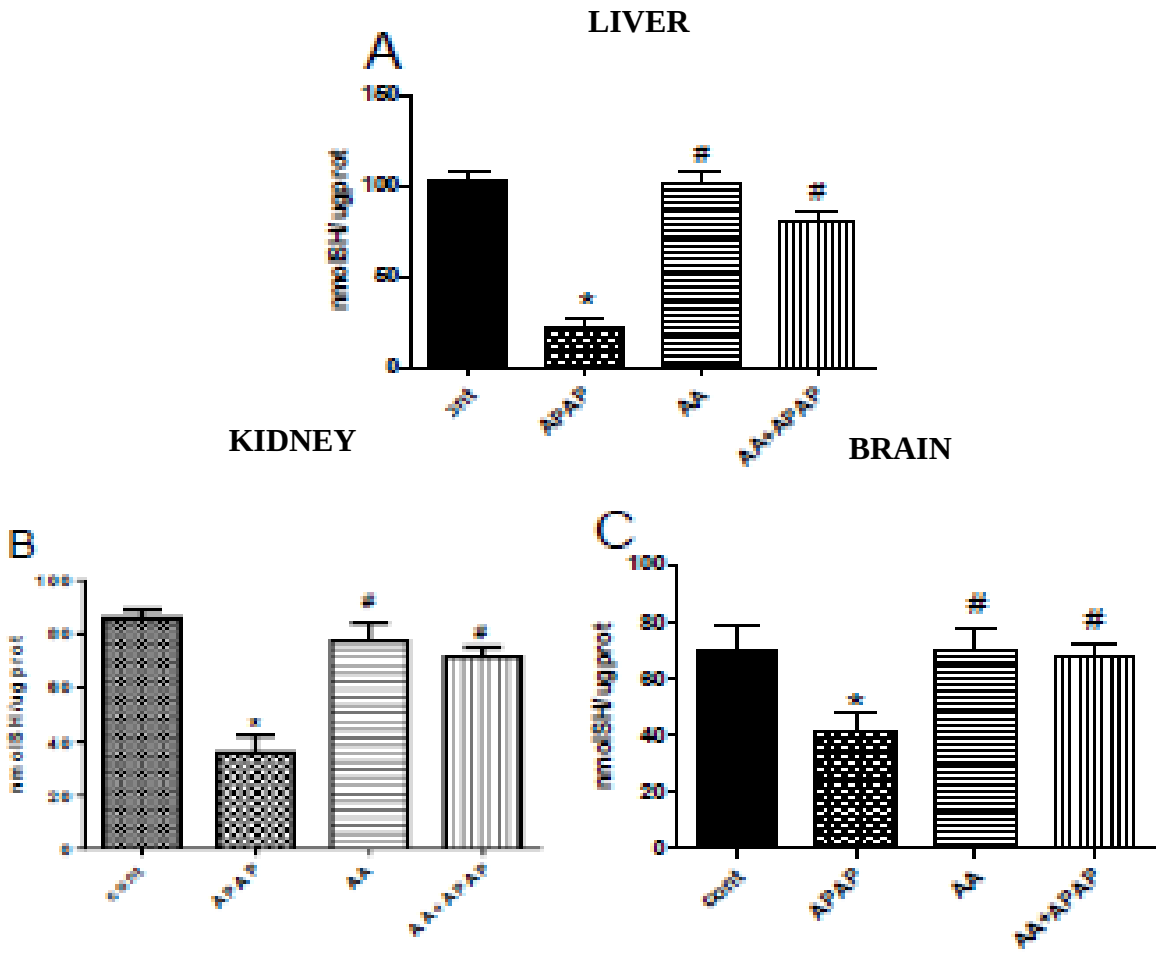

Figure 8: Effect of APAP and AA in SHNP levels in mice tissues: (A) liver; (B) kidneys and (C) brain. Dates were expressed as mean ± SEM (n = 4-6) and significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant differences were indicated by *or# $p < 0.05$; where * denotes difference to control group and # differences to APAP group.

Figure 9:

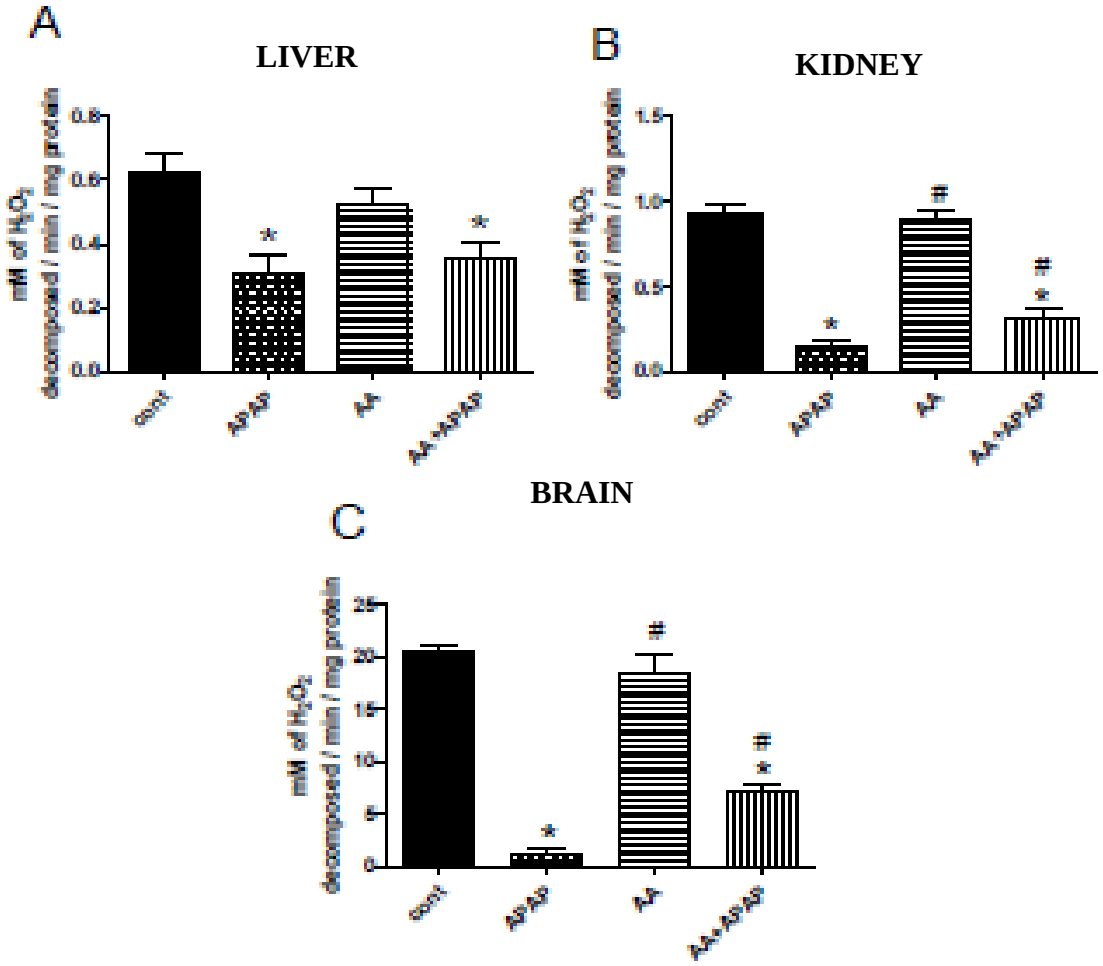

Figure 9: Effect of APAP and AA in Catalase levels in mice tissues: (A) liver; (B) kidneys and (C) brain. Dates were expressed as mean ± SEM (n = 4-6) and significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant differences were indicated by *or# $p < 0.05$ where * denotes difference to control group and # differences to APAP group.

Figure 10:

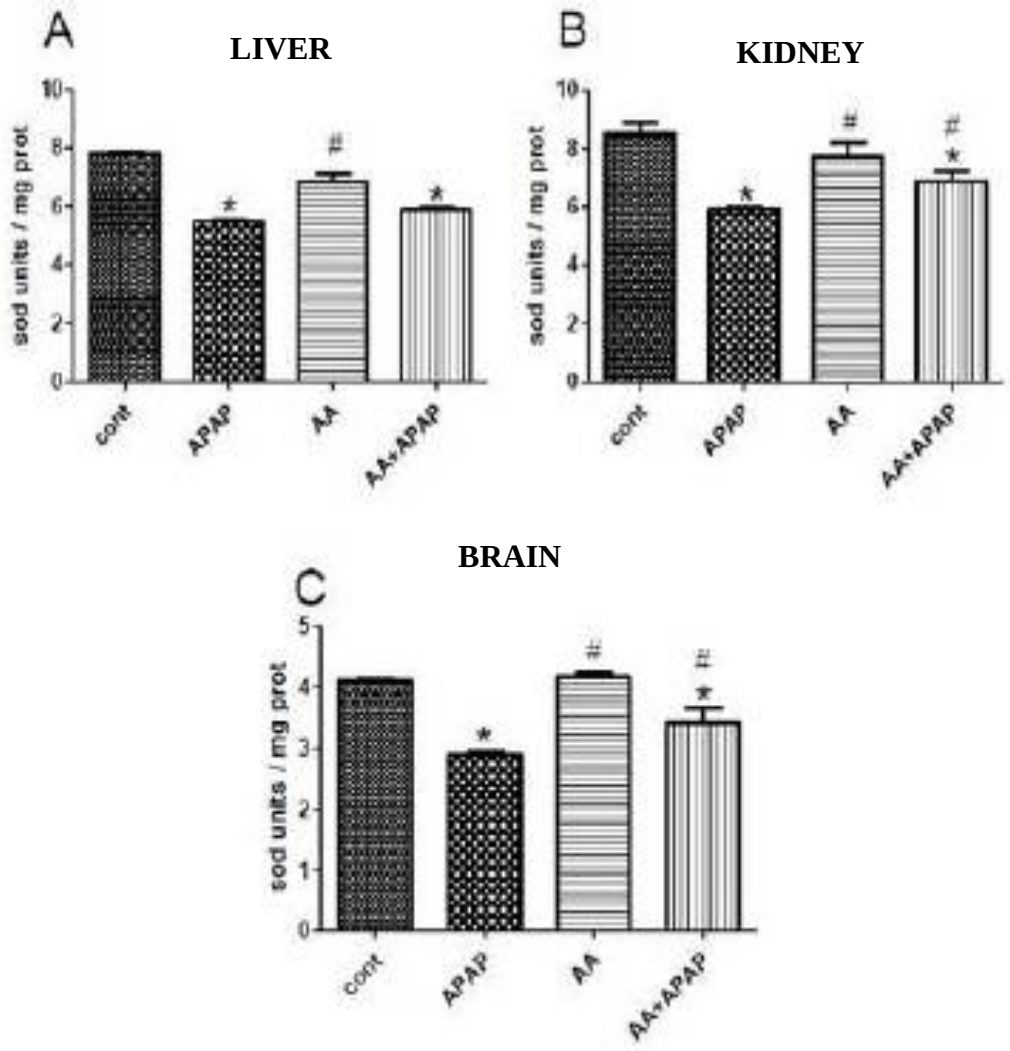

Figure 10: Effect of APAP and AA in SOD levels in mice tissues: (A) liver; (B) kidneys and (C) brain. Dates were expressed as mean ± SEM (n = 4-6) and significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant differences were indicated by *or# p <0.05; where * denotes difference to control group and # differences to APAP group.

DISCUSSION

There are times that men have been enjoying various plant species to obtain new therapies, drugs or formulas from natural compounds. The high levels and diversified poliphenols and flavonoids contents of medicinal plants have been usually associated with their therapeutic effects in several experimental models of diseases (Canadanovic-Brunet et al. 2005; Pandey & Rizvi 2009). Among the plants studied for medicinal purposes is possible to highlights the *Artemisia absinthium* specie, which was already shown to have significant amount of flavonoids, such as rutin, quercetin and kaempferol (Fakurazi et al. 2012). In fact, plants of the *Artemisia* genus have already been studied in many researches due to its antimicrobial , antinociceptive (Favero et al. 2014), anti-inflammatory and also hepatoprotective (Amat et al. 2010) properties. However, the effects of aqueous extract of AA against the hepatotoxicity induced by APAP were not yet investigated.

The aqueous extraction of AA used in this study presented high concentrations of polyphenols such as ferulic acid, caffeic acid and gallic acid. Some studies have been shown the protective effects of these polyphenols against the hepatotoxicity depicted by several drugs in mice. In this context, the ferulic acid was already described to have hepatoprotective effects against APAP toxicity (Krishnan et al. 2013), and caffeic acid was also effective to decrease the hepatotoxicity determined by carbon tetrachloride (Lee et al., 2008), and tamoxifen (Al Bukhari et al., 2009). Furthermore, the AA aqueous extract used here presented high concentrations of the flavonoids quercetin and kaempferol. The flavonoid glycoside kaempferol and C-8-C-beta-galactoside kaempferol, both presented in the plant Solanum elaeagnifolium and also in Clitoriaternanta (Nithianantham et al. 2011), were already reported to have protective effects against APAP induced toxicity in mice.

Therefore, the potential antioxidant effects of the AA aqueous extract observed in this study were probably linked to its high polyphenols and flavonoids content as described above. In fact, we

observed a significant potential of AA to decrease the basal and pro-oxidants induced lipid peroxidation even in low concentrations (10 to 25µg) in vitro. On the other hand, a significant DPPH• scavenging activity was observed only at 100µg of AA. The DPPH• scavenger analysis is a test commonly used to evaluate the ability of certain drugs, isolated compounds or extracts in neutralizing reactive species similar to hydroxyl radical (OH•) (Canadanovic-Brunet et al. 2005). Then, according to these results, we suggest that the mechanism related to the antioxidant capacity of AA aqueous extract could involve a possible metabolization of AA in the presence of biological tissues, as observed in in vitro experiments with mice tissue homogenates.

Regarding the *ex vivo* effects of the AA aqueous extract *per se*, we observed an almost complete absence of its toxicity in mice. It was observed in liver and brain mice tissues in a range of AA concentrations tested (from 1 to 100µg). However, the highest AA concentration tested (100µg) determined a significant decrease in kidneys NPSH levels. Is important to note that the antioxidant potential of AA aqueous extract to decrease the lipid peroxidation induced by Fe^{2+} was also observed in *ex vivo* experiments in mice liver (from 10 to 100µg) and kidneys (at 100µg). Furthermore, is important to emphasize that the aqueous extract of AA used in this work shows a lower toxicity threshold, which are in accordance with the concept that an aqueous extraction is generally the safest choice for animal or human treatments in comparison to other polar or non-polar solvents extraction of plants (Adedapo et al. 2007). Antioxidant potential associated with a low toxicity exhibited by AA was similar to the observed with other medicinal plants such as *Cyperusarticulatus* (S. Datta et al. 2013), *Feronialimonia* (Hanchinalmath & Londonkar 2014), *Clitoriaternatea* (Nithianantham et al. 2011;P. Datta et al. 2013), *Psidiumguajava* (Uboh et al., 2010) and *Orthosiphonstamineus* (Yam et al. 2010).

Taking into account the *in vitro* and also the *ex vivo* effects *per se* of the AA aqueous extract, we chosen the concentration of 10µg/µL of the AA to analyze its possible hepatoprotective effects

against APAP toxicity in a mice experimental model. The APAP concentration chosen (600mg/Kg of mice) was based in previous observations of an extensive hepatotoxicity with a low capacity of liver tissue regeneration (Carvalho et al. 2013; Rudraiah et al. 2014). The damage determined by this dosage was also previously associated to an inhibition of cell replication cycle and of the cyclin D1 protein expression (Craciunescu et al. 2012; Yang et al. 2012).

In fact, this APAP overdose lead to an excessive NAPQI metabolite formation, which lead to an increased ROS levels and also to a depletion of cellular antioxidant systems capacity of detoxification (mainly of GSH), contributing to an oxidative stress status establishment (Zhao et al. 2002, Carvalho et al. 2013). In line with this, we observed a significant increase in lipid peroxidation in all the analyzed mice tissue in response to APAP toxicity. Moreover a depletion of NPSH, GSH as a major compound, and a decrease in the antioxidant enzymes SOD e CAT activities were also observed. Is important to note that the AA aqueous extract was effective in minimize the oxidative damage depicted by APAP intoxication. Taken together, these results emphasize the antioxidant potential of AA aqueous extract that were also observed *in vitro* and *ex vivo* without APAP.

In general, our results reported a significant hepatotoxicity determined by APAP and potential protective effects of AA aqueous extract. In addition to oxidative damage, APAP increased the hepatic transaminase enzymes AST and ALT activities in serum, which suggest damage in hepatocyte cells structures. Further, APAP intoxication also decreased the MTT reduction capacity in mice liver and brain. The capacity of MTT reduction is a measure commonly used to infer the mitochondrial function and consequently the cellular viability, since that MTT is reduced to formazan by oxireductase family enzymes activities, mainly dehydrogenase enzymes, which are founded specially in tissue mitochondria (Bajt et al. 2004, Al-Belooshi et al. 2010). Therefore, we observed mitochondrial dysfunctions depicted by APAP intoxication, which were already reported in literature (Baliga et al. 2010, Yan et al. 2010). Both the changes in serum transaminases and the MTT reduction

activity determined by APAP intoxication were counteracted by AA aqueous extract pre-treatment (for four weeks previous APAP injection). Therefore, we suggest that a possible link between the antioxidant effects of AA with biological tissue (with or without APAP intoxication) and a prevention of mitochondrial dysfunction could be aspects. However, more studies are needed to highlight this hypothesis.

CONCLUSION

From this study we can conclude that the aqueous extract of AA had hepatoprotective properties against APAP induced toxicity, which are possible due to its high poliphenolic and flavonoids contents. In general, we suggest that the hepatoprotetive effects of AA against APAP intoxication were related to its antioxidant potential *in vitro* and *ex vivo*. Finally, further studies are needed to highlight the mechanism of AA aqueous extract action, especially its effects on mitochondrial dysfunction prevention.

CONFLICT OF INTEREST

The authors declare that there was no conflict of interest.

REFERENCES

Adedapo, a a, Omoloye, O. a & Ohore, O.G., 2007. Studies on the toxicity of an aqueous extract of the leaves of Abrus precatorius in rats. *The Onderstepoort journal of veterinary research*, 74(September 2006), pp.31–36.

Al-Belooshi, T. et al., 2010. Acetaminophen-induced Mitochondrial Oxidative Stress in Murine J774.2 Monocyte Macrophages. *American Journal of Biomedical Sciences*, 2(2), pp.142–154.

Amat, N., Upur, H. & Blažeković, B., 2010. In vivo hepatoprotective activity of the aqueous extract of Artemisia absinthium L. against chemically and immunologically induced liver injuries in mice. *Journal of Ethnopharmacology*, 131, pp.478–484.

Bajt, M.L. et al., 2004. Acetaminophen-induced oxidant stress and cell injury in cultured mouse hepatocytes: protection by N-acetyl cysteine. *Toxicological sciences : an official journal of the Society of Toxicology*, 80, pp.343–349.

Baliga, S.S. et al., 2010. Acetaminophen reduces mitochondrial dysfunction during early cerebral postischemic reperfusion in rats. *Brain Research*, 1319, pp.142–154. Available at: http://dx.doi.org/10.1016/j.brainres.2010.01.013.

Bertolini, A. et al., 2006. Paracetamol: New vistas of an old drug. *CNS Drug Reviews*, 12(3), pp.250–275.

Bessems, J.G. & Vermeulen, N.P., 2001. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. *Critical reviews in toxicology*, 31(1), pp.55–138.

Bunchorntavakul, C. & Reddy, K.R., 2013. Acetaminophen-related hepatotoxicity. *Clinics in liver disease*, 17(4), pp.587–607, viii. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24099020 [Accessed October 14, 2014].

Canadanovic-Brunet, J.M. et al., 2005. Free-radical scavenging activity of wormwood (Artemisia absinthium L) extracts. *Journal of the Science of Food and Agriculture*, 85(2), pp.265–272. Available at: http://doi.wiley.com/10.1002/jsfa.1950 [Accessed May 28, 2014].

Carolina, A. et al., 2015. Ac ce p te d cr t. *Biochemical Pharmacology*, 2. Available at: http://dx.doi.org/10.1016/j.bcp.2015.01.013.

Carvalho, N.R. et al., 2013. New therapeutic approach: diphenyl diselenide reduces mitochondrial dysfunction in acetaminophen-induced acute liver failure. *PloS one*, 8(12), p.e81961. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3859582&tool=pmcentrez&rendertype=abstract [Accessed October 14, 2014].

Chen, W. et al., 1998. Oxidation of acetaminophen to its toxic quinone imine and nontoxic catechol metabolites by baculovirus-expressed and purified human cytochromes P450 2E1 and 2A6. *Chemical Research in Toxicology*, 11(4), pp.295–301.

Chin, J.H., Hussin, a. H. & Ismail, S., 2009. Hepatoprotective effect of Orthosiphon stamineus Benth against acetaminophen intoxication in rats. *Journal of Natural Remedies*, 9, pp.177–184.

Craciunescu, O. et al., 2012a. Evaluation of antioxidant and cytoprotective activities of Arnica montana L. and Artemisia absinthium L. ethanolic extracts. *Chemistry Central Journal*, 6(1), p.97. Available at: Chemistry Central Journal.

Craciunescu, O. et al., 2012b. Evaluation of antioxidant and cytoprotective activities of Arnica montana L. and Artemisia absinthium L. ethanolic extracts. *Chemistry Central journal*, 6(1), p.97. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3472325&tool=pmcentrez&rendertype=abstract.

D'Mello, P. & Rana, M., 2010. Hepatoprotective activity of Psidium guajava extract and its phospholipid complex in paracetamol induced hepatic damage in rats. *International Journal of Phytomedicine*, 2, pp.85–93.

Dai, G. et al., 2005. Retinoid X Receptor ␣ Regulates the Expression of Glutathione S -transferase Genes and Modulates Acetaminophen- Glutathione Conjugation in Mouse Liver. , 68(6), pp.1590–1596.

Dart, R.C. et al., 2006. Acetaminophen Poisoning : an Evidence-Based Consensus Guideline for Out-of-Hospital Management *. , pp.1–18.

Datta, P., Karthivashan, G. & Fakurazi, S., 2013. Hepatoprotective nature of phytoextracts against hepatotoxin induced animal models: A review. *Journal of Chemical and Pharmaceutical Research*, 5(7), pp.233–239.

Datta, S. et al., 2013. Hepatoprotective activity of Cyperus articulatus Linn. against paracetamol induced hepatotoxicity in rats. *Journal of Chemical and Pharmaceutical Research*, 5(1), pp.314–319.

Ellman, G.L., 1959. Tissue sulfhydryl groups. *Archives of biochemistry and biophysics*, 82(1), pp.70–77.

Enomoto, A. et al., 2001. High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity associated with decreased expression of ARE-regulated drug metabolizing enzymes and antioxidant genes. *Toxicological Sciences*, 59, pp.169–177.

Fakurazi, S., Sharifudin, S.A. & Arulselvan, P., 2012. Moringa oleifera hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. *Molecules (Basel, Switzerland)*, 17(7), pp.8334–50. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22781444 [Accessed June 5, 2014].

Farrell, G.C., Unit, S.L. & Hospital, W., 1997. Drug-induced hepatic injury. , 12, pp.242–250.

Favero, F.D.F. et al., 2014. Artemisia annua L .: evidence of sesquiterpene lactones ' fraction antinociceptive activity. , pp.1–11.

Glatt, H. & Meinl, W., 2004. Pharmacogenetics of soluble sulfotransferases (SULTs). *Naunyn-Schmiedeberg's Archives of Pharmacology*, 369(1), pp.55–68.

Gonzalez, F.J., 2005. Role of cytochromes P450 in chemical toxicity and oxidative stress: Studies with CYP2E1. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*, 569(1-2), pp.101–110.

Grassi, D., Desideri, G. & Ferri, C., 2010. Flavonoids: antioxidants against atherosclerosis. *Nutrients*, 2, pp.889–902.

Hanchinalmath, J. V & Londonkar, R., 2014. I S S N 2278 – 4357 Anti Hepatotoxicity Studies Of Crude Extract Of Feronia Limonia In Ccl 4 Induced Toxicity. , 3(7), Pp.1536–1546.

Hawton, K. et al., 2004. UK legislation on analgesic packs: before and after study of long term effect on poisonings. *BMJ (Clinical research ed.)*, 329(October), p.1076.

Jaeschke, H. et al., 2002. Mechanisms of Hepatotoxicity. , 176, pp.166–176.

Jain, M. et al., 2011. Cytotoxicity evaluation and hepatoprotective potential of bioassay guided fractions from Feronia limmonia Linn leaf. *Asian Pacific Journal of Tropical Biomedicine*, 1, pp.443–447.

Kalsi, S.S. et al., 2011. Does cytochrome P450 liver isoenzyme induction increase the risk of liver toxicity after paracetamol overdose? *Open Access Emergency Medicine*, 3(July), pp.69–76.

Kim, H. et al., 2001. Differential induction of rat hepatic cytochromes P450 3A1, 3A2, 2B1, 2B2, and 2E1 in response to pyridine treatment. *Drug Metabolism and Disposition*, 29(3), pp.353–360.

Kis, B., Snipes, J. a & Busija, D.W., 2005. Acetaminophen and the cyclooxygenase-3 puzzle: sorting out facts, fictions, and uncertainties. *The Journal of pharmacology and experimental therapeutics*, 315(1), pp.1–7.

Krenzelok, E.P. & Royal, M. a, 2012. Confusion: acetaminophen dosing changes based on NO evidence in adults. *Drugs in R&D*, 12(2), pp.45–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3585765&tool=pmcentrez&rendertype=abstract.

Krishnan, D.N. et al., 2013. Hepatoprotective and antioxidant potential of ferulic acid against acetaminophen-induced liver damage in mice. *Comparative Clinical Pathology*, 22, pp.1177–1181.

Leung, R., Plomley, R. & Czarny, D., 1992. Paracetamol anaphylaxis. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*, 22(9), pp.831–833.

Moyer, A.M. et al., 2011. Acetaminophen-NAPQI hepatotoxicity: a cell line model system genome-wide association study. *Toxicological sciences : an official journal of the Society of Toxicology*, 120(1), pp.33–41. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3044203&tool=pmcentrez&rendertype=abstract [Accessed October 15, 2014].

Mutlib, A.E. et al., 2006. Kinetics of acetaminophen glucuronidation by UDP-glucuronosyltransferases 1A1, 1A6, 1A9 and 2B15. Potential implications in acetaminophen-induced hepatotoxicity. *Chemical Research in Toxicology*, 19(5), pp.701–709.

Nithianantham, K. et al., 2011. Hepatoprotective potential of clitoria ternatea leaf extract against paracetamol induced damage in mice. *Molecules*, 16, pp.10134–10145.

Pandey, K.B. & Rizvi, S.I., 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative medicine and cellular longevity*, 2(5), pp.270–278.

Phaneendra, P. & Kumar, M.R., 2011. Hepatoprotective Herbs : An Overview. , 3(0974), Pp.105–111.

Rudraiah, S. et al., 2014. Tolerance to Acetaminophen Hepatotoxicity in the Mouse Model of Autoprotection Is Associated with Induction of Flavin-containing Monooxygenase-3 (FMO3) in Hepatocytes.

Saska, S. et al., 2009. Cloridrato de tramadol / paracetamol no controle da dor pós-operatória em cirurgias de terceiros molares inclusos Tramadol / acetaminophen in the control of postoperative pain for impacted third molar surgery. , 5458, pp.99–106.

Saxena, M. & Shukla, S., 2012. Reversal of carbon tetrachloride-induced hepatic injury by aqueous extract of Artemisia absinthium in Sprague-Dawley rats. *Journal of environmental pathology, toxicology and oncology : official organ of the International Society for Environmental Toxicology and Cancer*, 31(4), pp.325–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23394445.

Schiødt, F. V et al., 1997. Acetaminophen toxicity in an urban county hospital. *The New England journal of medicine*, 337, pp.1112–1117.

Shaw, W., 2013. Evidence that Increased Acetaminophen use in Genetically Vulnerable Children Appears to be a Major Cause of the Epidemics of Autism , Attention Deficit with Hyperactivity , and Asthma. *Journal of Restorative Medicine*, 2, pp.1–16.

Uboh, F., 2010. Effect of Aqueous Extract of Psidium Guajava Leaves on Liver Enzymes, Histological Integrity and Hematological Indices in Rats. *Gastroenterology Research*, 3(1), pp.32–38.

Wu, L.C. et al., 2006. Antioxidant and antiproliferative activities of red pitaya. *Food Chemistry*, 95(2), pp.319–327.

Yam, M.F. et al., 2010. HPLC and anti-inflammatory studies of the flavonoid rich chloroform extract fraction of Orthosiphon stamineus leaves. *Molecules*, 15, pp.4452–4466.

Yan, H.M. et al., 2010. The oxygen tension modulates acetaminophen-induced mitochondrial oxidant stress and cell injury in cultured hepatocytes. *Toxicological Sciences*, 117(2), pp.515–523.

Yang, R. et al., 2012. High mobility group B1 impairs hepatocyte regeneration in acetaminophen hepatotoxicity. *BMC Gastroenterology*, 12, p.45.

Zamin, I. et al., 2002. A import??ncia do ??ndice AST/ALT no diagn??stico da esteatohepatite n??o-alco??lica. *Arquivos de Gastroenterologia*, 39(1), pp.22–26.

Zhao, P., Kalhorn, T.F. & Slattery, J.T., 2002. Selective mitochondrial glutathione depletion by ethanol enhances acetaminophen toxicity in rat liver. *Hepatology*, 36, pp.326–335.

LEGENDES OF FIGURES

Figure 1: Chemical Structure of APAP.

Figure 2: APAP's Metabolism.

Figure 3: High performance liquid chromatography phenolics and flavonoids profile of aqueous extract of AA and ethanolic extracts of AA.Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ferulic acid (peak 5), epicatechin (peak 6), rutin (peak 7), quercitrin (peak 8), isoquercitrin (peak 9), quercetin (peak 10) and kaempferol (peak 11).

Figure 4: DPPH scavenger activity of AA aqueous extract. Data are expressed as mean ± S.E.M. (n = 4-6) and significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant differences were indicated by * $p < 0.05$; where * denotes difference to control group.

Figure 5: Effect of APAP and AA in serum ALT (A) and AST (B) enzymes of mice. Dates were expressed as mean ± SEM (n = 4-6) and significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant differences were indicated by *or# $p < 0.05$; where * denotes difference to control group and # differences to APAP group.

Figure 6: Effect of APAP and AA in TBARS levels in mice tissues: (A) liver; (B) kidneys and (C) brain. Dates were expressed as mean ± SEM (n = 4-6) and significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant differences were indicated by *or# p <0.05; where * denotes difference to control group and # differences to APAP group.

Figure 7: Effect of APAP and AA in MTT levels in mice tissues: (A) liver; (B) kidneys and (C) brain. Dates were expressed as mean ± SEM (n = 4-6) and significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant differences were indicated by *or# p <0.05; where * denotes difference to control group and # differences to APAP group.

Figure 8: Effect of APAP and AA in SHNP levels in mice tissues: (A) liver; (B) kidneys and (C) brain. Dates were expressed as mean ± SEM (n = 4-6) and significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant differences were indicated by *or# p <0.05; where * denotes difference to control group and # differences to APAP group.

Figure 9: Effect of APAP and AA in Catalase levels in mice tissues: (A) liver; (B) kidneys and (C) brain. Dates were expressed as mean ± SEM (n = 4-6) and significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant differences were

indicated by *or# p <0.05 where * denotes difference to control group and # differences to APAP group.

Figure 10: Effect of APAP and AA in SOD levels in mice tissues: (A) liver; (B) kidneys and (C) brain. Dates were expressed as mean ± SEM (n = 4-6) and significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The significant differences were indicated by *or# p <0.05 where * denotes difference to control group and # differences to APAP group.

4. CONCLUSÃO:

4.1. Conclusão Geral

A partir deste trabalho foi possível estudar os principais componentes presentes no extrato aquoso de AA. Estes componentes fenólicos e flavonoides são descritos na literatura como possuidores de propriedades antioxidantes.

Os ensaios realizados in vitro, usando o extrato aquoso de AA juntamente com o radical DPPH confirmam o potencial antioxidante dos compostos citados anteriormente. E, por conseguinte, confirmam essas propriedades no extrato aquoso de AA. Já os ensaios *ex vivo*, utilizando uma escala de doses de 1 a 100 µG/µL, realizados com a finalidade de estudar o efeito per se do extrato AA mostraram a ausência de toxicidade do extrato.

Posteriormente, no modelo de tratamento onde os animais foram divididos em grupos distintos e pré- tratados com AA ou APAP na dose de 600mg/Kg os resultados foram satisfatórios e concordantes com os objetivos do estudo. O grupo que foi pré- tratado com o extrato AA conseguiu minimizar os efeitos induzidos pelo APAP. Este dado vai de encontro a outros estudos sobre o estresse oxidativo causado pelo APAP no tecido hepático. E além disso, reforçam as evidências que apontam para as propriedades hepato protetoras do extrato de AA.

4.2. Conclusões Específicas

O ensaio DPPH realizado no extrato aquoso de AA confirma o efeito antioxidante *in vitro* da planta. Ainda, o método TBARS demonstra a capacidade do extrato aquoso de AA em minimizar um dano induzido com pró oxidante. O aumento dos níveis séricos das enzimas ALT e AST corrobora a idéia de que APAP causa danos hepáticos relevantes (Zamin et al. 2002).

Nos ensaios *ex vivo*, os níveis de NPSH, MTT, DFCH-DA e TBARS tiveram seus níveis menos depletados em relação ao grupo que recebeu apenas o APAP. Este dado nos mostra que o tratamento com AA foi capaz de prevenir os danos causados pelo APAP no tecido hepático.

Na determinação da atividade das enzimas SOD e CAT os grupos tratados com o extrato aquoso AA apresentaram valores maiores do que o grupo que recebeu apenas o APAP. Este dado indica que o APAP causa uma depleção ou consumo nos níveis enzimáticos das mesmas, enquanto que o extrato de AA foi capaz de minimizar tal efeito.

Com esses resultados é possível concluir que o extrato aquoso de AA possui propriedades antioxidantes benéficas que funcionam na minimização do dano hepático induzido por APAP.

5. PERSPECTIVAS:

Tendo em vista os resultados obtidos neste trabalho, as perspectivas para trabalhos posteriores são:

Determinar o mecanismo provável pelo qual o extrato Aquoso de Artemísia Absinthium foi capaz de prevenir a hepatotoxicidade induzida por APAP. Através de forma antioxidante via inibição da formação da espécie reativa NAPQI ou através da prevenção das defesas antioxidantes endógenas.

Avaliar os parâmetros bioquímicos descritos e estudados neste trabalho em diferentes tipos de modelos de intoxicação. Avaliar o dano hepático em um estudo sub crônico com concentrações maiores de APAP. Ou ainda avaliar a extensão da hepatotoxicidade no uso crônico do APAP, o qual ocorre com alguns pacientes que fazem uso continuado do mesmo (Hawton et al. 2004).

Investigar a disfunção mitocondrial causada pelo APAP e o efeito do extrato de Artemísia Absínthium na mesma. Avaliar tal disfunção nos diferentes modelos de intoxicação: sub agudo, agudo e crônico. Desta maneira tentar elucidar os mecanismos que relacionam a cronicidade, o grau de intoxicação e a consequente extensão da lesão associados a disfunção mitocondrial.

6. REFERÊNCIAS:

Aebi, H. (1984). Catalase in Vitro. Method Enzym**.**105:121-126.

Amat, N., Upur, H. & Blažeković, B., 2010. In vivo hepatoprotective activity of the aqueous extract of Artemisia absinthium L. against chemically and immunologically induced liver injuries in mice. *Journal of Ethnopharmacology*, 131, pp.478–484.

Babot, Z.; Cristofol, R.; Sunol, C. (2005). Excitotoxic death induced by released glutamate in depolarized primary cultures of mouse cerebellar granule cells is dependent on GABAA receptors and niflumic acid-sensitive chloride channels. Eur J Neurosci.21:103–112

Bertolini, A. et al., 2006. Paracetamol: New vistas of an old drug. *CNS Drug Reviews*, 12(3), pp.250–275.

Bessems, J.G. & Vermeulen, N.P., 2001. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. *Critical reviews in toxicology*, 31(1), pp.55–138.

Ellman, GL. (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82:70–77

Carolina, A. et al., 2015. Ac ce p te d cr t. *Biochemical Pharmacology*, 2. Available at: http://dx.doi.org/10.1016/j.bcp.2015.01.013.

Chen, W. et al., 1998. Oxidation of acetaminophen to its toxic quinone imine and nontoxic catechol metabolites by baculovirus-expressed and purified human cytochromes P450 2E1 and 2A6. *Chemical Research in Toxicology*, 11(4), pp.295–301.

Craciunescu, O. et al., 2012a. Evaluation of antioxidant and cytoprotective activities of Arnica montana L. and Artemisia absinthium L. ethanolic extracts. *Chemistry Central Journal*, 6(1), p.97. Available at: Chemistry Central Journal.

Fakurazi, S., Sharifudin, S.A. & Arulselvan, P., 2012. Moringa oleifera hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. *Molecules (Basel, Switzerland)*, 17(7), pp.8334–50. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22781444 [Accessed June 5, 2014].

Farrell, G.C., Unit, S.L. & Hospital, W., 1997. Drug-induced hepatic injury. , 12, pp.242–250.

Glatt, H. & Meinl, W., 2004. Pharmacogenetics of soluble sulfotransferases (SULTs). *Naunyn-Schmiedeberg's Archives of Pharmacology*, 369(1), pp.55–68.

Gonzalez, F.J., 2005. Role of cytochromes P450 in chemical toxicity and oxidative stress: Studies with CYP2E1. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*, 569(1-2), pp.101–110.

Grassi, D., Desideri, G. & Ferri, C., 2010. Flavonoids: antioxidants against atherosclerosis. *Nutrients*, 2, pp.889–902.

Hawton, K. et al., 2004. UK legislation on analgesic packs: before and after study of long term effect on poisonings. *BMJ (Clinical research ed.)*, 329(October), p.1076.

Hinz, B., Cheremina, O. & Brune, K., 2008. Acetaminophen (paracetamol) is a selective cyclooxygenase-2 inhibitor in man. *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 22(2), pp.383–390

Kim, H. et al., 2001. Differential induction of rat hepatic cytochromes P450 3A1, 3A2, 2B1, 2B2, and 2E1 in response to pyridine treatment. *Drug Metabolism and Disposition*, 29(3), pp.353–360.

Kis, B., Snipes, J. a & Busija, D.W., 2005. Acetaminophen and the cyclooxygenase-3 puzzle: sorting out facts, fictions, and uncertainties. *The Journal of pharmacology and experimental therapeutics*, 315(1), pp.1–7.

Glatt, H. & Meinl, W., 2004. Pharmacogenetics of soluble sulfotransferases (SULTs). *Naunyn-Schmiedeberg's Archives of Pharmacology*, 369(1), pp.55–68.

Kalsi, S.S. et al., 2011. Does cytochrome P450 liver isoenzyme induction increase the risk of liver toxicity after paracetamol overdose? *Open Access Emergency Medicine*, 3(July), pp.69–76.

Kis, B., Snipes, J. a & Busija, D.W., 2005. Acetaminophen and the cyclooxygenase-3 puzzle: sorting out facts, fictions, and uncertainties. *The Journal of pharmacology and experimental therapeutics*, 315(1), pp.1–7.

Leung, R., Plomley, R. & Czarny, D., 1992. Paracetamol anaphylaxis. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*, 22(9), pp.831–833.

Misra, H. et al., 2014. Extraction of Artemisinin , an Active Antimalarial Phytopharmaceutical from Dried Leaves of Artemisia annua L ., Using Microwaves and a Validated HPTLC-Visible Method for Its Quantitative Determination. , 2014.

Moslemi, H.R. et al., 2012. Antimicrobial Activity of Artemisia absinthium Against Surgical Wounds Infected by Staphylococcus aureus in a Rat Model. *Indian journal of microbiology*, 52(4), pp.601–4. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3516646&tool=pmcentrez&rendertype=abstract [Accessed June 5, 2014].

Mutlib, A.E. et al., 2006. Kinetics of acetaminophen glucuronidation by UDP-glucuronosyltransferases 1A1, 1A6, 1A9 and 2B15. Potential implications in acetaminophen-induced hepatotoxicity. *Chemical Research in Toxicology*, 19(5), pp.701–709.

Oga , Seize ; Camargo , Marcia Maria de A .; BATISTUZZO , José Antonio O. Toxicology Fundamentals . 3. ed. São Paulo : Atheneu , 2008 .

Saxena, M. & Shukla, S., 2012. Reversal of carbon tetrachloride-induced hepatic injury by aqueous extract of Artemisia absinthium in Sprague-Dawley rats. *Journal of environmental pathology, toxicology and oncology : official organ of the International Society for Environmental Toxicology and Cancer*, 31(4), pp.325–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23394445.

Schmidt, L.E., 2005. Age and paracetamol self-poisoning. *Gut*, 54(5), pp.686–690.

Schillie, S.F. et al., 2009. Medication Overdoses Leading to Emergency Department Visits Among Children. *American Journal of Preventive Medicine*, 37(3), pp.181–187. Available at: http://dx.doi.org/10.1016/j.amepre.2009.05.018.

Tariq, K. a et al., 2009. Anthelmintic activity of extracts of Artemisia absinthium against ovine nematodes. *Veterinary parasitology*, 160(1-2), pp.83–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19070963 [Accessed June 3, 2014].

Vollhardt, K. Peter., 2007. Organic Chemistry - Structure and Function - 6th Ed .

Wu, C. et al., 1979. The chromatin structure of specific genes: I. Evidence for higher order domains of defined DNA sequence. *Cell*, 16(4), pp.797–806.

Zamin, I. et al., 2002. A import??ncia do ??ndice AST/ALT no diagn??stico da esteatohepatite n??o-alco??lica. *Arquivos de Gastroenterologia*, 39(1), pp.22–26.