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BRUNA SANTOS DA SILVA

AVALIAÇÃO DOS EFEITOS DA DIETILCARBAMAZINA SOBRE OS MECANISMOS REGULATÓRIOS DO NF-κB NA LESÃO HEPATOCELULAR INDUZIDA PELO ALCOOLISMO

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Dra. Teresinha Gonçalves da Silva – UFPE

Dedico à minha mãe
Maria (in memoriam).

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RESUMO

A indução da expressão gênica mediada pelo NF-κB foi descrita na patogênese da doença hepática alcoólica (DHA). Dietilcarbamazina (DEC) é um fármaco derivado da piperazina com propriedades anti-inflamatórias. O presente estudo foi desenvolvido para avaliar o efeito de DEC na via do NF-κB na inflamação hepática induzida pelo alcoolismo. Quarenta camundongos machos C57BL/6 foram divididos igualmente em quatro grupos: 1) grupo controle (C), que recebeu apenas água, 2) grupo tratado com DEC, que recebeu 50 mg/kg durante um período de 12 dias (DEC50), 3) o grupo alcoólico (EtOH), submetido ao consumo crônico de álcool e 4) o grupo alcoólico tratado com DEC (EtOH50), submetido ao consumo crônico de álcool e tratado com DEC. Fragmentos de fígado foram analisados por meio de microscopia de luz, imunohistoquímica e testes de western blot para avaliar vários mecanismos envolvidos na lesão hepática induzida pelo etanol, incluindo a peroxidação lipídica, marcadores inflamatórios e a ativação de fatores de transcrição. A análise histológica do grupo alcoólico mostrou dano hepatocelular evidente que foi reduzido no grupo alcóolico tratado com DEC. Os resultados da imunohistoquímica e do western blot mostraram expressão elevada de marcadores inflamatórios como MDA, TNF-α, IL-1β, COX-2 e iNOS nos hepatócitos do grupo EtOH. No entanto, pouca imunopositividade para estes marcadores foi detectada após tratamento com DEC. No grupo de EtOH a ativação do fator de transcrição NF-kB foi observada através de um aumento na expressão de ambos, NF-κB e pNF-κB, em hepatócitos. Esta expressão foi significativamente reduzida nos fígados do grupo EtOH50. A expressão da proteína IκBα foi medida para determinar se a ativação do NF-κB seria resultado da degradação da IκBα. Observou-se que a expressão desta enzima era baixa no grupo EtOH, enquanto que os animais tratados com DEC tinha uma expressão elevada de IκBα. Os resultados do presente estudo indicam que a DEC atenua a lesão hepática alcoólica, em parte, pela inibição da ativação do NF-κB e por suprimir a indução de genes dependentes do NF-κB.

Palavras-chave: Dietilcarbamazina, alcoolismo, dano hepático, marcadores inflamatórios, fatores de trancição, NF-κB

ABSTRACT

Induction of NF-κB-mediated gene expression has been identified in the pathogenesis of alcoholic liver disease (ALD). Diethylcarbamazine (DEC) is a piperazine derivative drug with anti-inflammatory properties. The present study was designed to evaluate the effect of DEC on NF-κB pathways undergoing alcoholism induced hepatic inflammation. Forty male C57BL/6 mice were divided equally into four groups: 1) control group (C), which received only water, 2) DEC- treated group, which received 50 mg/kg during a 12 day period (DEC50), 3) the alcoholic group (EtOH), submitted to chronic alcohol consumption and 4) the alcohol-DEC treated group (EtOH50), submitted to chronic alcoholism consumption plus DEC treatment. Liver fragments were analyzed using light microscopy, immunohistochemical and western blot tests to evaluate various mechanisms involved in ethanol-induced hepatic damage, including lipid peroxidation, inflammatory markers and activation of transcription factors. Histological analysis of the alcoholic group showed evident hepatocellular damage which was reduced in the alcoholic DEC-treated group. Immunohistochemistry and western blot results showed elevated expression of inflammatory markers such as MDA, TNF-α, IL-1β, COX-2 and iNOS in hepatocytes of the EtOH group. However, low immunopositivity for these markers was detected following DEC treatment. In the EtOH group the activation of transcription factor NF-κB was observed by an increase in the expression of both NF-κB and pNF-κB in hepatocytes. This expression was significantly reduced in the EtOH50 livers. Protein expression of Iκβα was measured to determine whether activation of NF-κB might be the result of Iκβα degradation. It was observed that expression of this enzyme was low in the EtOH group, while animals treated with DEC had a high expression of Iκβα. The results of the present study indicate that DEC alleviates alcoholic liver injury, in part by the inhibiting activation of NF-κB and by suppressing the induction of NF-κB-dependent genes.

Keywords: Diethylcarbamazine, alcoholism, hepatic injury, inflammatory markers, transcription factors, NF-κB

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

ACC	Acetil-CoA carboxilase
ACL	Citratoliase
ALD	Álcool desidrogenase
ALDH	Aldeído desidrogenase mitocondrial
AMPK	Proteína kinase adenosina monofosfato ativada
AP-1	Proteína ativadora 1
ATP	Adenosina trifosfato
DEC	Dietilcarbamazina
ERK 1/2	Receptor ativado de kinase 1/2
DNA	Ácido desoxiribonucleico
CCl_4	Tetracloreto de carbono
COX-2	Ciclooxigenase 2
CYP2E1	Citocromo P450 2E1
DHA	Doença hepática alcoólica
FAS	Ácido graxo sintase
HSC	Células estreladas hepáticas
IAP	Proteína inibitória da apoptose
iNOS	Óxido nítrico sintase induzível
IL-1β	Interleucina 1β
IL-6	Interleucina 6
JNK	c-jun-N-terminal kinase
LPS	Lipopolissacarídeo
MCP-1	Proteína quimiotática de monócito 1
MFB	Miofibroblastos
MIP-2	Proteína inflamatória de macrófago 2
mRNA	Ácido ribonucleico mensageiro
NAD	Nicotinamida adenina dinucleótideo
NDPH	Nicotinamida adenina dinucleotídeo fosfato
NF-kB	Fator nuclear kappa B
PPARs	Receptores ativado por proliferadores de peroxissomos
PPARα	Receptor ativado por proliferadores de peroxissomos alfa
PPARγ	Receptor ativado por proliferadores de peroxissomos gama

RHD	Domínio de homologia REL
ROS	Espécies reativas de oxigênio
SCD	Esterol-CoA desnaturase
SOCS	Supressor de sinalização de citocinas
SREBP	Proteína de ligação ao elemento regulador de esterol
STAT3	Sinal transdutor e ativador de transcrição 3
TGFβ	Fator de transformação de crescimento beta
TLR4	Receptor Toll-like 4
TNF-α	Fator de necrose tumoral alfa

SUMÁRIO

1 INTRODUÇÃO

O alcoolismo é a causa mais frequente de doenças hepáticas nos países ocidentais (SHERLOCK & DOOLEY, 2002). Segundo a organização mundial de saúde, em todo o mundo, a cada ano o uso nocivo do álcool mata 2,5 milhões de pessoas, incluindo 320.000 jovens entre 15 e 29 anos de idade (WHO, 2011).

O efeito tóxico do álcool tem impacto em muitos órgãos, entretanto, o fígado como o sítio primário do metabolismo do etanol é o maior alvo (LIEBER, 2000, 2005; KARINCH *et al*., 2008). A doença hepática alcóolica (DHA) é considerada um processo patológico complexo e multifatorial que envolve o estresse oxidativo, inflamação e a síntese excessiva de ácidos graxos (DING *et al*., 2010). A progressão da doença envolve várias moléculas inflamatórias como interleucinas, citocinas, moléculas de adesão e fatores de transcrição, como o fator de transcrição nuclear kappa-B (NF-κB) (ACHUR *et al*., 2010; BALLAS *et al*., 2012; ROCHA *et al*., 2012).

O NF-κB está envolvido na inflamação e na resposta imune (BAEUERLE & BALTIMORE, 1996) e é ativado por oxidantes, interleucinas e citocinas como a interleucina 1β (IL-1β) e o fator de necrose tumoral alfa (TNF-α) (BARNES & KARIN, 1997) que apresentam um papel importante na inflamação e no desenvolvimento da DHA. A IL-1β e o TNF–α ativam a degradação e fosforilação das proteínas IκB, permitindo a entrada do NF-κB no núcleo que ativa a transcrição de vários genes incluindo o da ciclooxigenase 2 (COX-2) e da sintase de oxido nítrico induzível (iNOS) que são consideradas importantes mediadores no recrutamento de células inflamatórias (BHASKARAN *et al*., 2010; ARIAS-SALVATIERRA *et al*., 2011).

O consumo do álcool também inibe a oxidação de ácidos graxos nos hepatócitos via inativação do receptor ativado por proliferadores de peroxissomos alfa (PPAR-α), um receptor nuclear hormonal que controla a transcrição de genes envolvidos no transporte e oxidação dos ácidos graxos (YU *et al*., 2003; WAGNER *et al*., 2011). O etanol também pode afetar a atividade de enzimas envolvidas no metabolismo do ácido graxo por inibir a proteína kinase AMP-ativada (AMPK) (VIOLLET *et al*., 2009). A inibição do NF-κB é capaz de restaurar a atividade dos receptores ativados por

proliferadores de peroxissomos (PPARs) e a expressão de genes ligados a esse receptor (SERRANO-MARCO *et al*., 2012).

A identificação do NF-κB como um fator chave na patogênese da inflamação sugere este fator de transcrição como alvo terapêutico podendo ser eficaz no tratamento dessas doenças (TAK & FIRESTEIN, 2001). Uma variedade de fármacos usados para tratar doenças inflamatórias em humanos apresenta efeito na via do NF-κB (YAMAMOTO & GAYNOR, 2001).

O tratamento da doença hepática pode apresentar resultados positivos, mas também traz consideráveis efeitos negativos. O curso clínico da lesão hepática pode ser melhorado com o uso de compostos naturais com propriedades anti-oxidantes (resveratrol), corticóides e agentes anti-TNF-α (pentoxifilina, infliximab, o etanercept). No entanto, mais dados clínicos são necessários para padronizar ou combinar estes tratamentos porque essas drogas na verdade, aumentaram o risco de mortalidade, de infecção e de morte dos pacientes (GAO E BATALLER, 2011; BRUHA *et al*, 2012).

A dietilcarbamazina (DEC) é um derivado da piperazina, utilizada eficazmente há mais de 50 anos no tratamento da filariose linfática. Além disso, ela também apresenta propriedades anti-inflamatórias, em parte devido a alterações no metabolismo do ácido araquidônico (NORÕES *et al.,* 1997).

Poucos estudos tem focado o papel da DEC na patofisiologia da inflamação. Alguns estudos clínicos relataram que a DEC reduziu os sintomas da asma brônquica devido aos seus efeitos anti-inflamatórios (THIRUVENGADAM *et al.*, 1974; MAIZELS & DENHAM, 1992). Confirmando tais resultados, Queto et al. (2010) observou a ação da DEC no bloqueio da inflamação eosinofílica pulmonar em camundongos. De acordo com Gonzalez et al. (1994) ratos com inflamação hepática induzida por tetracloreto de carbono (CCl_4) apresentaram uma evidente redução do dano hepático após o tratamento com DEC nas doses de 25 e 50 mg/kg. Rocha et al. (2012) demonstraram que o tratamento com DEC (50 mg/kg) inibiu o dano hepático e reduziu o infiltrado inflamatório induzidos pelo consumo crônico de etanol.

Dessa forma, o presente trabalho se propõe a investigar a ação anti-inflamatória da DEC na via do NF–κB em um modelo de lesão hepática crônica induzida pelo álcool, como uma possível alternativa no tratamento da doença hepática alcoólica.

2 JUSTIFICATIVA

Apesar do profundo impacto econômico e de saúde da DHA, pouco progresso tem sido obtido no tratamento de pacientes com esta condição clínica grave. Não existem ferramentas modernas de diagnóstico para avaliar a susceptibilidade individual para o desenvolvimento da DHA, e a patogênese dessa doença em seres humanos não é completamente compreendida. Como consequência, nenhum novo fármaco para DHA foi desenvolvido com êxito desde os anos 1970, momento em que o uso de corticosteroides foi proposto para o tratamento da hepatite alcoólica grave (HELMAN *et al.*, 1971; GAO & BATALLER, 2011). O pobre progresso terapêutico no domínio da DHA tem, em parte, resultado da falta de modelos experimentais com a forma avançada da doença e da dificuldade de realização de ensaios clínicos em doentes com desejo compulsivo.

O dano presente em DHA envolve a indução genes de citocinas, de proteases e oxidases que se propagam através de respostas que envolvem as vias de sinalização do NF-κB (NANJI *et al*., 1999). A definição dos mecanismos envolvidos na inflamação do fígado e morte celular durante a DHA abrirá novas perspectivas para o desenvolvimento deste processo e deve oferecer potenciais alvos para intervenções terapêuticas. Uma variedade de fármacos usados para tratar doenças inflamatórias em humanos apresenta efeito na via do NF-κB. Alguns efeitos dos corticoides, usados no tratamento de várias doenças inflamatórias como DAH, são provavelmente mediados pela inibição da ativação do NF-κB (YAMAMOTO & GAYNOR, 2001).

A DEC tem sido a droga de escolha para o tratamento da filariose linfática desde 1947 (OTTENSEN, 2000; FLORENCIO E PEIXOTO, 2003). No entanto, apesar de mais de 60 anos de seu uso, seu mecanismo de ação ainda permanece pouco esclarecido. Porém, sabe-se que a DEC apresenta propriedades anti-inflamatórias, como um resultado de sua interferência no metabolismo do ácido araquidônico (MAIZELS & DENHAM, 1992).

Diante da necessidade de novos fármacos eficazes para o tratamento da DHA, a DEC por suas propriedades anti-inflamatórias e seu potencial farmacológico pouco explorado, se mostra como possível alternativa, sendo necessários dessa forma, estudos mais aprofundados das suas propriedades e do seu mecanismo de ação, objetivos deste trabalho.

3 OBJETIVOS

3.1 Objetivo Geral

Caracterizar o efeito do tratamento *in vivo* da DEC (50 mg/kg) na via de ativação do NF-κB durante a inflamação hepática induzida pelo alcoolismo.

3.2 Objetivos Específicos

- ✓ Avaliar a expressão do NF-κB, pNF-κB e de sua enzima regulatória IκBα;
- ✓ Identificar a expressão de marcadores inflamatórios regulados pelo NF-κB como TNF-α, IL-1β, COX-2 e iNOS;
- ✓ Avaliar a homeostase lipídica através de marcadores como o MDA, PPARα, AMPK, pAMPK;
- ✓ Investigar a indução da via MAPK pela análise da expressão de JNK e c-jun;
- ✓ Analisar a ativação de outros fatores de transcrição como o PPARγ.

CAPÍTULO I

4 REVISÃO DA LITERATURA

4.1 ESTRUTURA E FUNÇÃO HEPÁTICA

O fígado é a maior glândula do corpo humano. Situado no quadrante superior direito da cavidade abdominal, logo abaixo do diafragma, está subdividido em quatro lobos - direito, esquerdo, quadrado e caudado - dos quais os dois primeiros constituem a quase totalidade (Figura 1 A) (GARTNER & HIATT, 2007).

O principal tipo celular presente no fígado e que é responsável pela maior parte de suas funções metabólicas é o hepatócito (célula parenquimatosa). Os hepatócitos compreendem 65% das células do fígado e 80% do volume hepático (WANLESS, 1999). São células poligonais que estão bem próximas umas das outras, agrupadas em placas interconectadas, os lóbulos hepáticos (Figura 1 B). Estas células apresentam variações em suas propriedades estruturais, histoquímicas e bioquímicas, dependendo de sua localização nos lóbulos hepáticos (GARTNER & HIATT, 2007; JUNQUEIRA & CAR NEIRO, 2008).

O parênquima hepático é organizado em "placas" de hepatócitos, visto em cortes microscópicos como cordões de células disposta radialmente a partir de uma veia central (Figura 1C). Entre os cordões de hepatócitos estão os sinusóides vasculares (capilares hepáticos) (Figura 1C). Os sinusóides são revestidos por células endoteliais, que demarcam um espaço extrasinusoidal (espaço de Disse), no qual sobressaem as microvilosidades dos hepatócitos. Ocasionalmente, fibras nervosas amielínicas e células armazenadoras de gordura, estreladas, (também denominadas células de Ito, ou células estreladas**)** foram observadas neste espaço**.** Os macrófagos residentes, denominados células de Kupffer**,** estão associados às células de revestimento endotelial dos sinusóides (Figura 2). Além disso, alguns tipos de linfócitos podem ser encontrados no fígado, como parte de um mecanismo de defesa contra agentes infecciosos (ISSELBACHER & PODOLSKY, 1991; GARTNER & HIATT, 2007).

O suprimento de sangue para o fígado deriva de duas fontes: da veia porta e da artéria hepática; a primeira fornece aproximadamente 75% do fluxo total de 1.500 mL/min, sangue pouco oxigenado e rico em nutrientes proveniente das vísceras abdominais. O restante, deriva da artéria hepática, que fornece sangue rico em oxigênio.

Os pequenos ramos da vênula portal terminal e da arteríola hepática terminal, entram nos ácinos da tríade portal e então fluem através dos sinusóides entre placas de hepatócitos (BEERS & BERKOW, 2001).

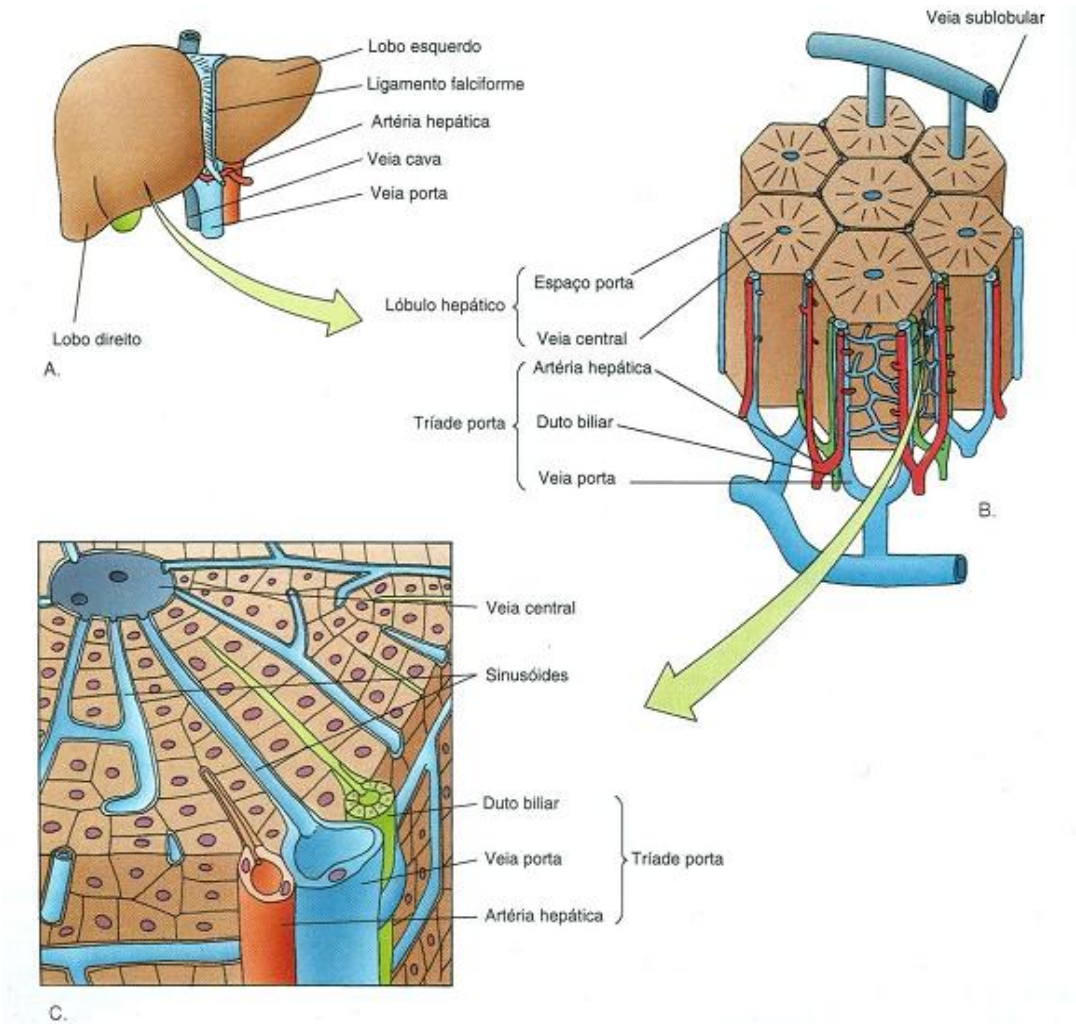

Figura 1. Desenho esquemático do fígado. (A) anatomia macroscópica do fígado; (B) lóbulos hepáticos; (C) Parte de um lóbulo hepático em maior aumento (GARTNER & HIATT, 2007).

A troca de nutrientes ocorre através dos espaços de Disse, que separam os hepatócitos do revestimento sinusoidal poroso. O fluxo sinusoidal se mistura nas vênulas hepáticas terminais que coalescem e finalmente, formam a veia hepática, responsável pelo transporte de todo o sangue eferente para a veia cava inferior. O fígado também é drenado por um suprimento rico de vasos linfáticos. É comum ocorrer interferência no suprimento sanguíneo hepático na cirrose e em outras doenças crônicas que se manifestam geralmente por hipertensão portal (BEERS & BERKOW, 2001).

O fígado apresenta um papel crucial na manutenção da homeostase metabólica. Suas funções incluem processamento de aminoácidos, carboidratos, lipídeos e vitaminas, síntese de proteínas do soro, biotransformação de metabólitos circulantes, desintoxicação e excreção de resíduos de produtos endógenos (CRAWFORD, 1994). Além disso, as células de Kupffer fagocitam partículas estranhas presentes no sangue e hemácias não funcionantes (GARTNER & HIAT, 2007). O parênquima hepático está envolvido na resposta imune e em mudanças metabólicas. Estas mudanças metabólicas ocorrem em resposta a endotoxinas, citocinas, substâncias vasoativas ou a outros mediadores inflamatórios. Os hepatócitos podem expressar um rico repertório de receptores em sua superfície que assegura o envolvimento direto destes mediadores em processos celulares (DINARELLO, 1984; BAUMANN *et a*l., 1987; POMPOSELLI *et al*., 1988).

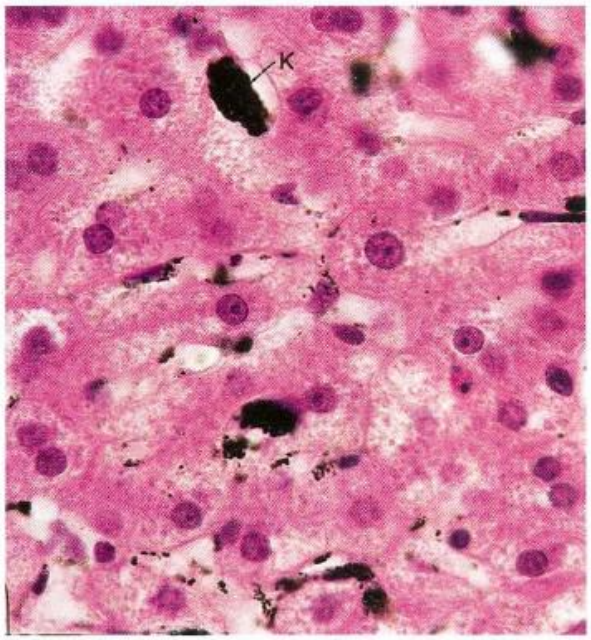

Figura 2. Fotomicrografia de fígado de cão mostrando as placas de hepatócitos, sinusóides e células de Kupffer contendo tinta nanquim (K) (540 X). (GARTNER & HIATT, 2007).

4.2 ETANOL

4.2.1 Aspectos Gerais

O etanol (CH_3CH_2OH), também chamado álcool etílico e, na linguagem corrente, simplesmente álcool, é uma substância orgânica obtida da fermentação de açúcares, hidratação do etileno ou redução a acetaldeído. Após administração oral, o etanol é rapidamente absorvido para corrente sanguínea a partir do estômago e intestino delgado. Níveis sanguíneos máximos ocorrem 30-90min após sua a ingestão quando o estômago está vazio. Atrasos no esvaziamento gástrico (devido à presença de alimentos) atrasam a absorção do etanol, uma vez que a absorção ocorre mais rapidamente a partir do intestino delgado do que a partir do estômago (HOLFORD, 1987) (Figura 3 B). Uma vez absorvido, o álcool é transportado para o fígado através da veia portal. Uma porção do álcool ingerido é metabolizada durante a sua passagem inicial através do fígado, o restante do álcool ingerido deixa o fígado, entra na circulação sistémica, e é distribuída ao longo dos tecidos do corpo (Figura 3 A) (WEATHERMON & CRABB, 1999).

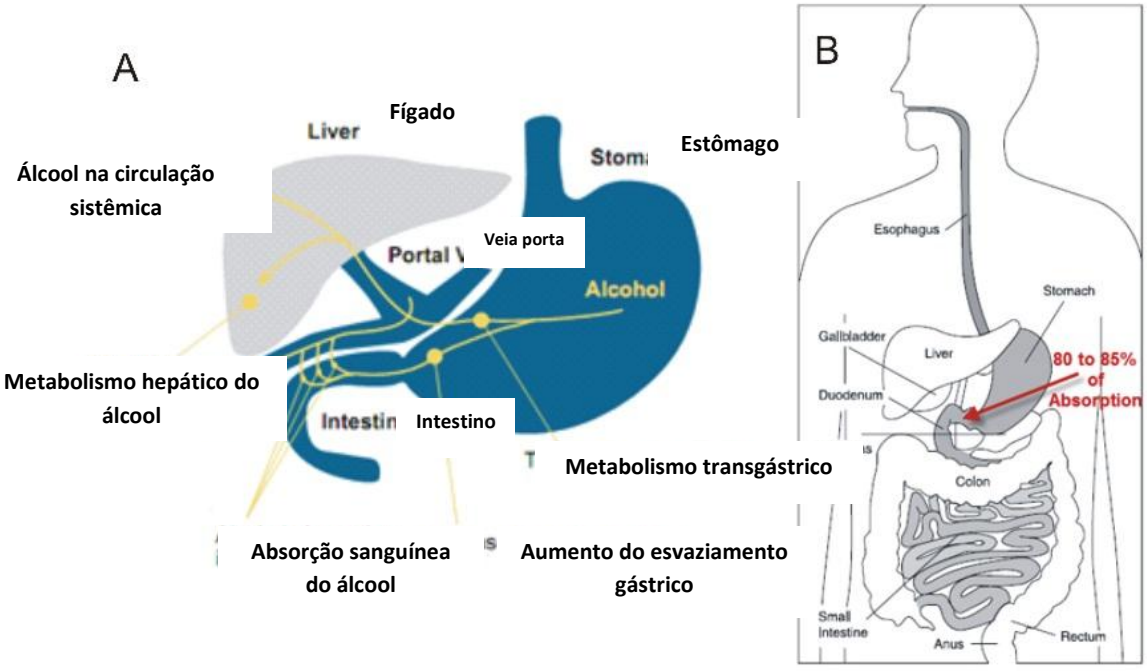

Figura 3. Absorção do etanol. (A) Representação esquemática do metabolismo de primeira passagem do etanol pelo fígado. (B) Locais de absorção do álcool no corpo (regiões escuras na imagem) (WEATHERMON & CRABB, 1999; HEAD, 2011).

4.2.2 Metabolismo

O primeiro passo no metabolismo oxidativo do álcool é efetuado por enzimas-chave, incluindo a álcool desidrogenase (ALD), citocromo P450 2E1 (CYP2E1), e a catalase. A ALD é a principal enzima oxidante, tendo uma elevada afinidade pelo álcool

e quebra o etanol no citoplasma (FREEMAN *et al.*, 2005). A CYP2E1 é utilizada por uma via diferente que é induzida pelo consumo crônico do álcool, e resulta na formação de acetaldeído nos microssomos. Uma terceira via do primeiro passo do metabolismo do etanol é mediada pela oxidação do etanol pela catalase nos peroxissomos (CRABB & LIANGPUNSAKUL, 2007).

O segundo passo, que é principalmente realizado pela aldeído desidrogenase mitocondrial (ALDH), é metabolizar o acetaldeído para acetato. Além disso, o acetaldeído pode ser metabolizado pela CYP2E1 através de uma via dependente de NADPH (sistema microssomal de oxidação do etanol) (KUNITOH *et al.*, 1997) (Figura 4). O acetato resultante é instável e espontaneamente se decompõe-se em água e gás carbônico (CO_2). Quando esses mecanismos oxidativos tornam-se oprimidos, o acetaldeído se acumula e exerce seus efeitos tóxicos. A natureza eletrofílica do acetaldeído (FREEMAN *et al.*, 2005) permite que ele se ligue e forme adutos, isto é, produtos químicos de ligação covalente, com proteínas, lipídeos, e DNA (NIEMELA, 2007). Adutos são amplamente patogênicos, pois prejudicam as funções de proteínas e lipídeos, e promovem danos e mutação ao DNA (TUMA & CASEY, 2003).

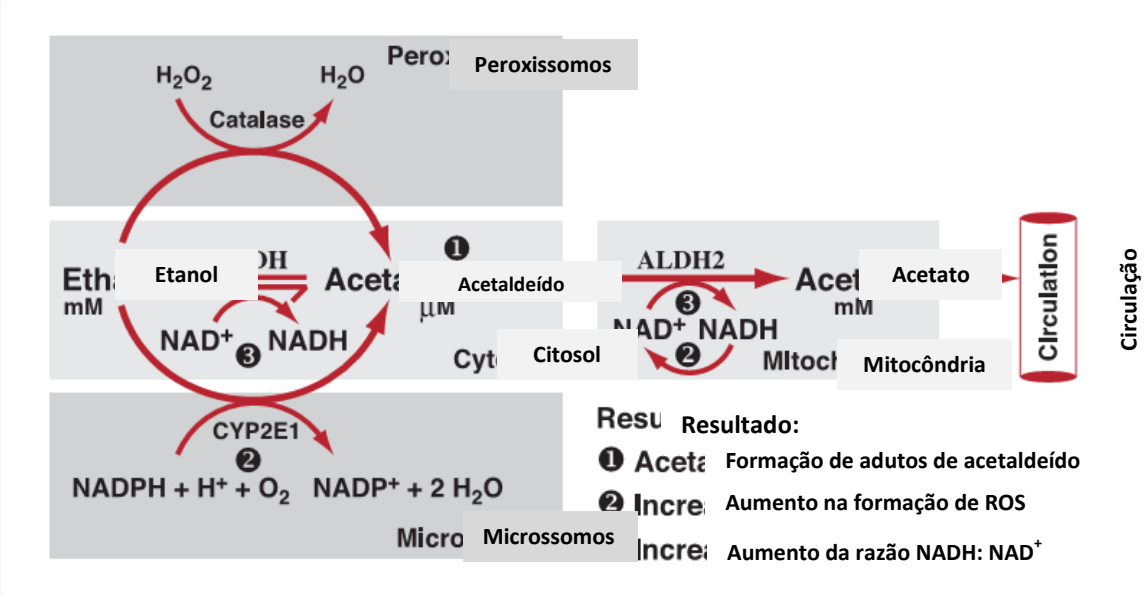

Figura 4. Vias oxidativas do metabolismo do etanol no fígado (ZAKHARI, 2006).

O álcool e seu metabolismo causam alterações na capacidade do fígado em eliminar várias substâncias. Assim, o metabolismo do álcool, afeta o estado redox do fígado e os níveis de glutationa, um antioxidante que impede que espécies reativas de

oxigênio (ROS) danifiquem as células. O termo "estado redox" refere-se às concentrações de duas substâncias nas células: nicotinamida adenina dinucleotídeo (NAD^+) e NAD^+ reduzido (NADH), que são necessários para o funcionamento de várias enzimas. O metabolismo do álcool pela ADH resulta na conversão de NAD^+ em NADH, aumentando assim os níveis de NADH do fígado. Níveis elevados de NADH, por sua vez, estimulam a geração de moléculas de gordura e interferem com a capacidade de outras enzimas hepáticas em quebrar moléculas de gordura e produzir glicose. Por meio destas alterações metabólicas, o metabolismo do álcool pode afetar substancialmente o metabolismo geral do corpo e seu funcionamento (WEATHERMON & CRABB, 1999).

4.3 DOENÇA HEPÁTICA ALCOÓLICA (DHA)

O alcoolismo representa uma das principais questões sociais e econômicas que o mundo enfrenta. Em todo o mundo, a cada ano o uso nocivo do álcool mata 2,5 milhões de pessoas, incluindo 320.000 jovens entre 15 e 29 anos de idade (WHO, 2011).

A doença hepática alcoólica (DHA) representa um espectro de sintomas clínicos e alterações morfológicas que variam de fígado gorduroso, inflamação hepática e necrose (hepatite alcoólica) à fibrose progressiva (cirrose alcoólica) (TOME & LUCEY, 2004) (Figura 5). Além disso, a manutenção do consumo excessivo de álcool favorece a progressão de outras doenças hepáticas, tais como hepatite crônica relacionada a vírus, além do risco aumentado de desenvolver carcinoma hepatocelular (MANDAYAM *et al.*, 2004; SAFDAR & SCHIFF, 2004).

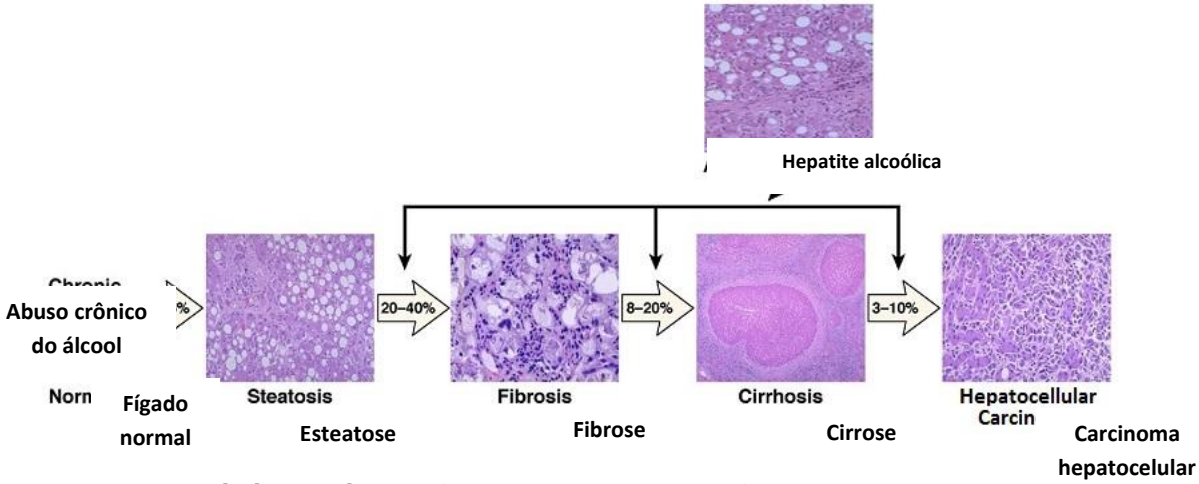

Figura 5. Espectro de doenças da DHA (GAO & BATALLER, 2011).

A dependência do álcool, por si só nem sempre é um pré-requisito para o desenvolvimento da DHA (BELLENTANI *et at.*, 1997). Pois, além da quantidade consumida de álcool, fatores genéticos e ambientais provavelmente também desempenham papel crucial no desenvolvimento da DHA (STEWART *et al.*, 2001; ROUAULT, 2003).

Embora uma relação dose-efeito entre a ingestão de álcool e a lesão hepática induzida pelo álcool tenha sido relatada, não há nenhuma quantidade definida de consumo de álcool que pode certamente prever o desenvolvimento de DHA (BECKER *et al.*, 1996). Na verdade, a maioria dos obesos desenvolve fígado gorduroso (cerca de 90%) (CRABB, 1999), porém apenas 10-35% desenvolvem hepatite e apenas 8-20% irão avançar para a cirrose (SORENSEN *et al.*, 1984; TELI *et al.*, 1995; BELLENTANI *et at.*, 1997).

Estudos preconizam que o consumo diário de álcool de cerca de 40–80 g/dia para homens e 20–40 g/dia para mulheres por 10–12 anos levará a quase 100% de chance de desenvolvimento da DHA (FUCHS *et al.*, 1995; BECKER *et al.*, 1996; THUN *et al.*, 1997).

O processo de lesão hepática é bastante complexo, resultando de desordens bioquímicas, genéticas, celulares, imunológicas e humorais em conexão com a ingestão e o metabolismo de quantidades excessivas de álcool (YIN *et al.*, 1999) (Figura 6).

Dentre os mecanismos associados à lesão hepática induzida pelo álcool, dados experimentais e humanos sugerem um papel principal para a citocromo P450 2E1 (CYP2E1), uma enzima microssomal que pode ser induzida 10-20 vezes pelo consumo crônico de álcool (WANG *et al.*, 2009). A CYP2E1 metaboliza o etanol a acetaldeído, uma molécula altamente tóxica e mutagênica, e aumenta o estresse oxidativo através da produção de espécies reativas de oxigênio (ROS) e peróxidos de lipídeos, tais como 4-hidroxi 2,3-nonenal, 4-hidroxi-2,3 - alqueno e malondialdeído (WU & CEDERBAUM, 2009). A indução da CYP2E1 está associada ao acúmulo de gordura (LU *et al.*, 2008), inflamação e fibrose (LIEBER, 2004) e lesões ao DNA (WANG *et al.*, 2009). Além disso, o consumo excessivo de álcool pode conduzir a um aumento portal-sistêmico da absorção de endotoxinas a partir de bactérias intestinais que contribuem para inflamação-necrose e progressão da fibrose através de vários mecanismos moleculares,

incluindo o TNF-α e do complexo CD14/receptor toll-like para produzir ROS via NADPH oxidase (ALTAMIRANO & BATALLER, 2011) (Figura 6).

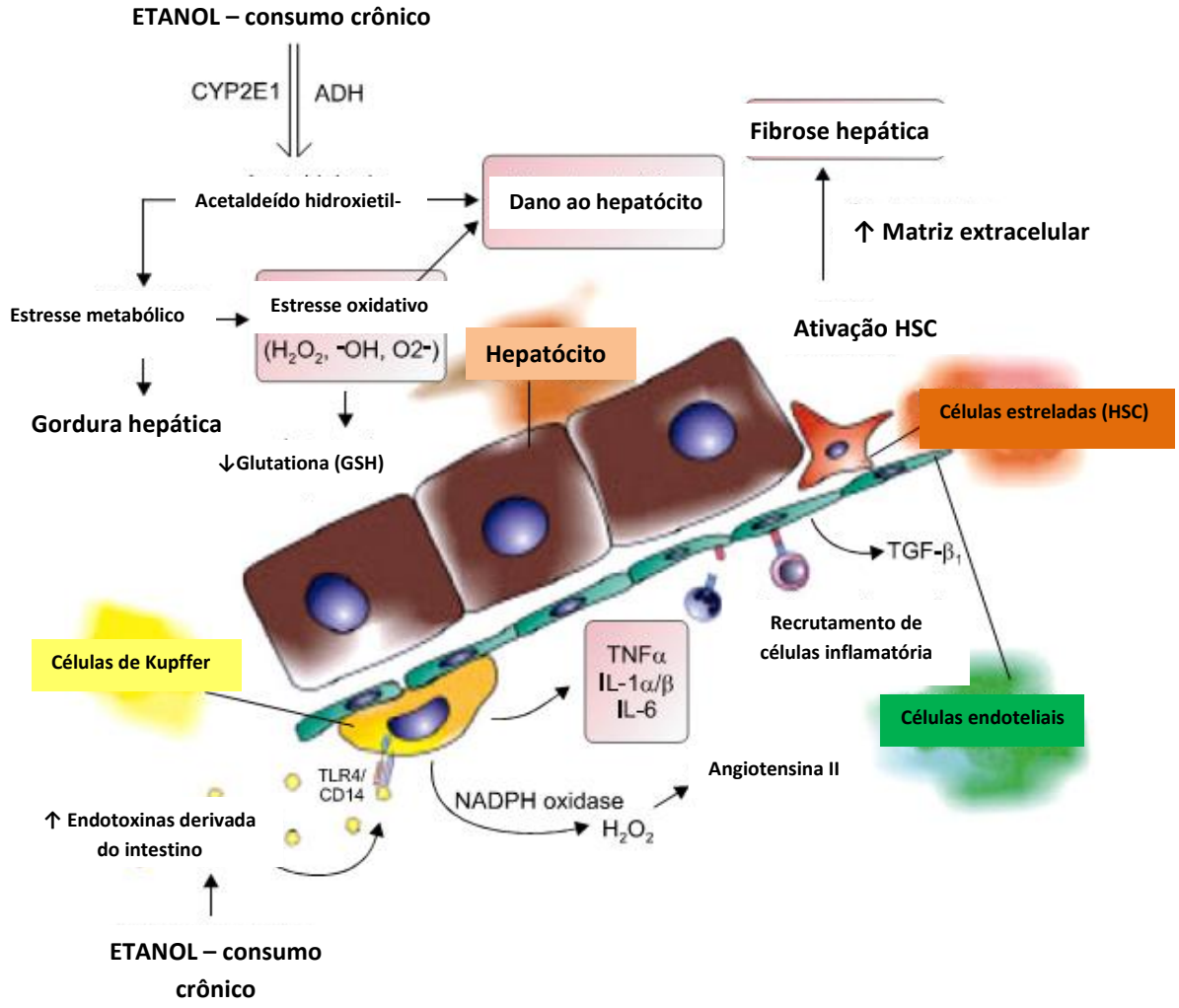

Figura 6. A patogênese da inflamação hepática induzida pelo álcool e o papel do sistema imune inato (CHAE, 2009).

Além disso, a ingestão de álcool aumenta a permeabilidade intestinal para uma variedade de substâncias que incluem endotoxinas bacterianas, tais com o lipopolissacarídeo (LPS) (TILG & DIEHL, 2000). Este por sua vez, 'sensibiliza' as células de kupffer através da ligação com o receptor CD14. Esta ligação ativa o NF-κB

que leva a transcrição exacerbada de citocinas pró-inflamatórias, tais como TNF-α, IL-1β, IL-6 e fator de crescimento transformante beta1 (TGFβ1) (HOEK & PASTORINO, 2004). Além da produção de citocinas, a ativação do NF-κB pode levar a transcrição de genes da ciclooxigenase 2 (COX-2) e da óxido nítrico sintase induzível (iNOS) que são considerados importantes mediadores no recrutamento de células inflamatórias (BHASKARAN *et al*., 2010; ARIAS-SALVATIERRA *et al*., 2011). A COX-2 medeia a síntese de prostaglandina durante a inflamação e no fígado sua expressão está relacionada com fenômenos inflamatórios presente em diferentes doenças crônicas (CHARIYALERTSAK *et al*., 2001; GIANNITRAPANI *et al*., 2009). iNOS é expressa nas células hepáticas em condições patológicas como cirrose e hepatite. Durante o processo de infecção e inflamação no fígado, o aumento na produção de óxido nítrico (NO) pela iNOS está relacionado com o dano hepático (MATSUI *et al*., 2011). Tais fatores irão contribuir para a inflamação, apoptose e fibrose, com a consequente progressão da doença.

4.4 ÁLCOOL E MAP KINASE

O LPS ativa os membros da família MAPK incluindo o receptor de kinase ativado 1 / 2 (ERK1 / 2), p38 e kinase c-jun-N-terminal (JNK) resultando na produção de TNF-α (SWEET, 1996). O alcoolismo crônico aumenta a ativação do ERK1 / 2 induzida pelo LPS e (KISHORE *et al*., 2002), assim como, ativa o JNK levando a fosforilação do c-jun que se liga ao sítio promotor de TNFα CRE/AP-1 (SWEET, 1996). Esses eventos contribuem para o aumento da produção de TNFα. A ativação do p38 contribui com a estabilidade do mRNA do TNFα via interação com o tristetrapolin (TTP) (MAHTANI *et al*., 2001) (Figura 7).

Álcool

Estabilidade do mRNA do TNF-α

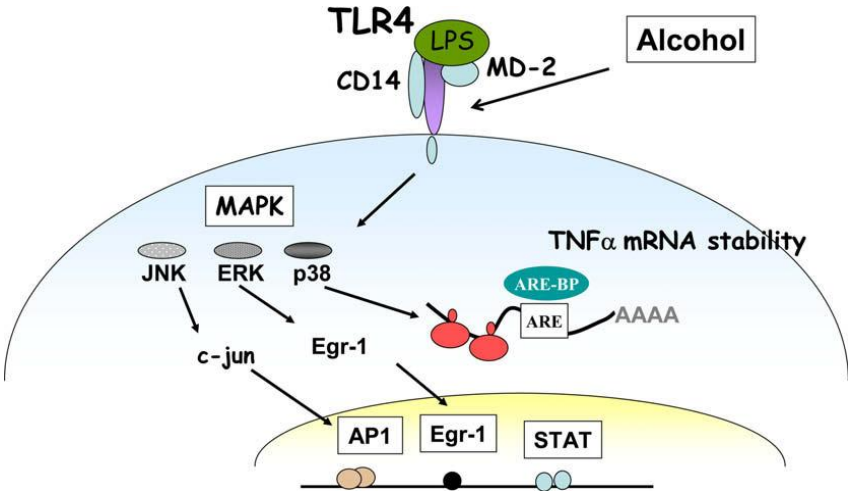

Figura 7. Via de sinalização da MAP quinase em macrófagos expostos ao etanol (MANDREKAR & SZABO, 2009).

4.5 FATORES DE TRANSCRIÇÃO INDUZIDOS PELO ÁLCOOL

Em resposta a exposição ao etanol, múltiplas vias de transdução de sinal são ativadas por diferentes receptores em vários tipos celulares do fígado, culminando em eventos nucleares envolvendo a ligação de fatores de transcrição aos elementos promotores de genes-alvo. O consumo do álcool regula de forma direta ou indireta o metabolismo lipídico por aumentar a expressão de genes envolvidos na síntese de ácidos graxos e suprimir genes relacionados com a oxidação lipídica, resultando em esteatose hepática. Fatores de transcrição que regulam o metabolismo lipídico como a proteína de ligação ao elemento regulador de esterol (SREBP) e o receptor ativado por proliferadores de peroxissomos alfa (PPARα), que participa da oxidação lipídica, têm um papel importante na ADH (MANDREKAR, 2007).

4.5.1 SREBPs

Os SREBPs são importantes fatores de transcrição que regulam a síntese de ácidos graxos e colesterol no fígado por ativar enzimas envolvidas nessas vias de biossíntese, como a adenosina trifosfato (ATP), citratoliase (ACL), acetil-CoA

carboxilase (ACC), ácido graxo sintase (FAS) e a esterol-CoA desnaturase (SCD) (HORTON *et al*., 2002)

O consumo de álcool pode aumentar a transcrição do SREBP-1c diretamente via acetaldeído ou indiretamente por ativar processos e fatores que estimulam sua expressão como resposta do retículo endoplasmático ao estresse celular, adenosina, endocanabinoides, sinalização de LPS via receptor Toll-like 4 (TLR4) (YOU *et al.*, 2002). O álcool também pode diminuir a regulação de fatores que diminuem a expressão do SREBP-1c como proteína kinase AMP-ativada (AMPK), adiponectina e o sinal transdutor e ativador de transcrição 3 (STAT3) (YOU *et al.*, 2004) (Figura 8).

4.5.2 PPARα

Os receptores ativados de peroxissomo de proliferação (PPARs) controlam a expressão de genes que atuam no metabolismo de lipídeos e carboidratos, na biologia vascular, no reparo tecidual, na proliferação e diferenciação celular e no dimorfismo sexual. PPARs compõem uma subfamília com três membros: PPAR alfa (PPARα), PPAR beta/delta (PPARβ/δ) e o PPAR gama (PPARγ) (MICHALIK *et al*., 2006).

O PPARα, um fator regulatório essencial na oxidação de ácidos graxos, apresenta um papel importante na esteatose hepática durante o alcoolismo (MANDREKAR, 2007). O consumo de álcool inibi a oxidação de ácidos graxos via inativação do receptor de PPARα, que controla a transcrição de genes envolvidos no transporte e na oxidação lipídica (YU *et al*., 2003; WAGNER *et al*., 2011). O acetaldeído, metabólito do etanol, inibi diretamente a ativação transcricional e a habilidade de ligação ao DNA do PPARα nos hepatócitos (GALLI *et al*., 2001). O alcoolismo também pode, indiretamente, inibir o PPARα por aumentar a regulação do citocromo P450 2E1 derivado do estresse oxidativo (LU *et al*., 2008) ou por diminuir a regulação de adiponectina e zinco, que ativam o PPARα (YOU *et al*., 2005; KANG *et al*., 2009) (Figura 8).

Na regulação do metabolismo lipídico associado aos fatores de transcrição, o etanol também pode afetar a atividade de enzimas envolvidas no metabolismo dos ácidos graxos por inibir o AMPK, que reduz o metabolismo lipídico e a gordura hepática (GAO & BATALLER, 2011).

O AMPK é uma enzima pertencente a família da proteína kinase e que apresenta um papel central na regulação do metabolismo lipídico por inibir a regulação de enzimas como a acetil-CoA carboxilase (ACC) e a 3-hidroxi-3-metilglutaril-CoA redutase (HARDIE, 1992; HARDIE *et al.*, 1998). O consumo de álcool inibe a atividade do AMPK no fígado, levando a diminuição da fosforilação e aumento da atividade da ACC (YOU et al., 2004) (Figura 8).

Além de atuar nos fatores de transcrição envolvidos na homeostase lipídica, o consumo de etanol também exerce efeitos na ativação de fatores de transcrição relacionados com a liberação de mediadores inflamatórios, que apresentam um papel importante no desenvolvimento da ADH. Esses fatores de transcrição são: a proteína ativadora 1 (AP-1), fator de resposta ao crescimento inicial 1 (Erg-1), receptor ativado por proliferadores de peroxissomos gama (PPARγ) e o fator de transcrição nuclear kappa B (NF-κB) (MANDREKAR, 2007).

4.5.3 AP-1

Os fatores de transcrição proteína ativadora - 1 (AP-1) são homodímeros e heterodímeros compostos de proteínas pertencentes às famílias Jun (c-Jun, Jun çB e JunD), Fos (c-Fos, FosB, Fra-1, Fra-2), JDP (JDP1, JDP2) e ATF. A ativação AP-1 regula a proliferação e morte celular através da indução de moduladores do ciclo celular tais como a ciclina D1 e o p53. O AP-1 é ativado por citocinas pró-inflamatórias, estresse oxidativo, fatores de crescimento e endotoxinas (SHAULIAN & KARIN, 2001). A ativação do AP-1 durante o alcoolismo crônico é importante porque esse fator de transcrição regula genes envolvidos na resposta inflamatória como o TNF-α, e o CD14 (WHEELER & THURMAN, 2003). O AP-1 também está envolvido com o desenvolvimento da fibrose hepática por regular a transcrição de metaloproteinases e colágeno tipo I (ARMENDARIZ-BORUNDA *et al*., 1994).

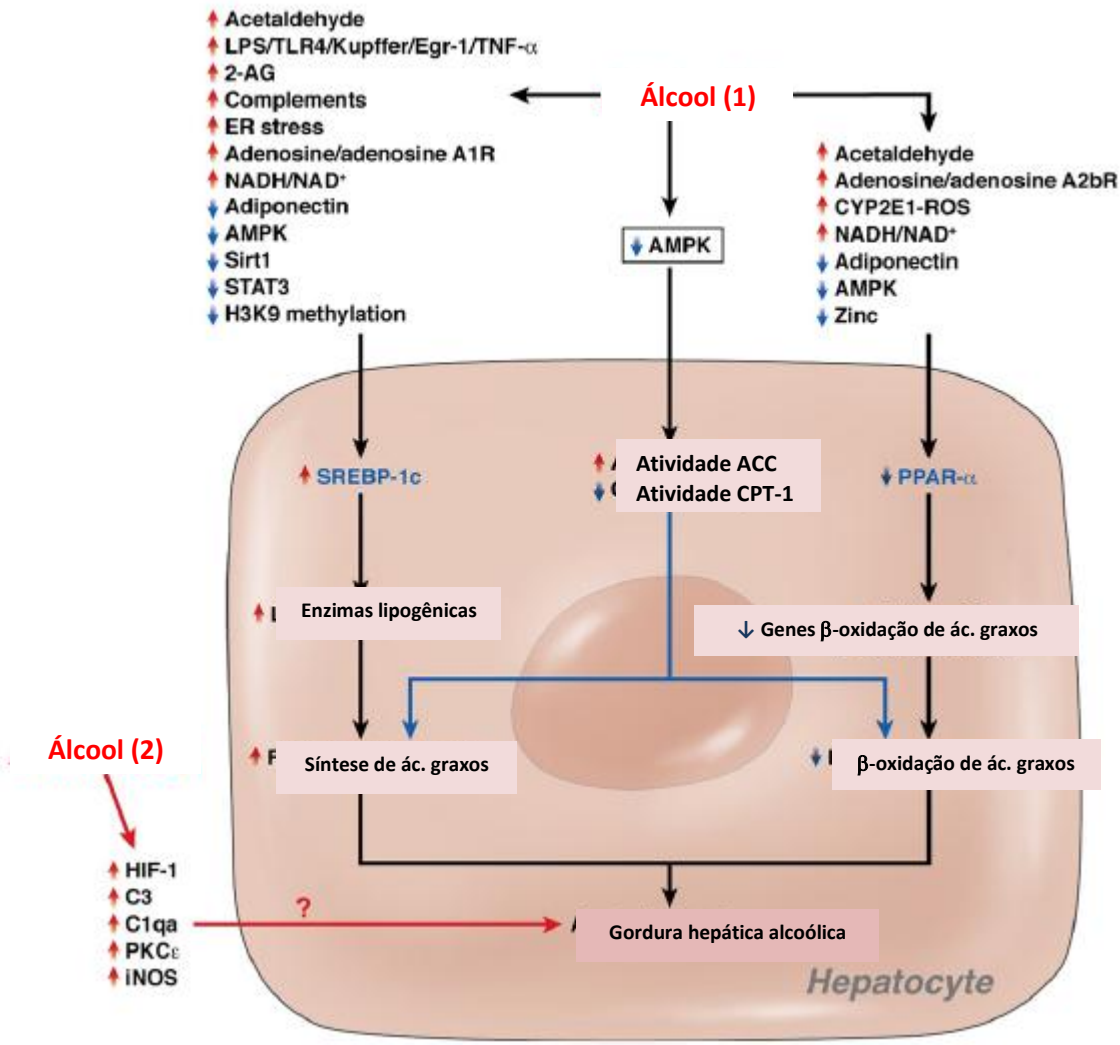

Figura 8. Mecanismos envolvidos no acúmulo de lipídeo hepático durante o consumo de etanol. (GAO & BATALLER, 2011).

4.5.4 Egr-1

Egr-1 é um fator de transcrição induzido em resposta ao estresse ambiental, regulado pela cascata de sinalização da MAPK e induzido pelo LPS. O Egr-1 é necessário para a indução da expressão de TNF-α, moléculas de adesão, fator de crescimento de fibroblasto básico, fator de transformação de crescimento beta (TGFβ), proteína quimiotática de monócito 1 (MCP-1) e proteína inflamatória de macrófago 2 (MIP-2) (YAN *et al.*, 2000). O aumento da expressão de Egr-1 foi dependente da ativação de ERK1/2 em células de Kupffer durante o alcoolismo crônico e a injúria hepática induzida pelo álcool foi bloqueada em camundongos *knockout* para o Egr-1,

indicando o papel da via ERK1/2-Egr-1 na patogênese da DHA (KISHORE *et al*., 2002; MCMULLEN *et al*., 2005).

4.5.5 PPARγ

O PPARγ é expresso em macrófagos e está implicado na resposta imune inata (DAYNES & JONES, 2002). Sua expressão está aumentada durante o desenvolvimento da esteatose hepática em resposta a dieta com alto teor lipídico, obesidade e exposição crônica ao etanol (VIDAL-PUIG *et al*., 1996; MEMON *et al*., 2000; RAHIMIAN *et al*., 2001; BOELSTERLI & BEDOUCHA, 2002). Durante a exposição ao álcool, a expressão do PPARγ foi observada nas células de Kupffer e nos hepatócitos (BOELSTERLI & BEDOUCHA, 2002) e o tratamento com agonista do PPARγ preveniu o desenvolvimento da esteatose e da inflamação (ENOMOTO *et al*., 2003).

4.5.6 NF-κB

O fator de transcrição nuclear kappa B (NF-κB) foi identificado por David Baltimore, em 1986 como uma molécula que, no núcleo, se liga ao promotor do gene da cadeia kappa de imunoglobulinas em células B (AGGARWAL, 2004). Sabe-se hoje, que o NF-κB no seu estado inativo está presente no citoplasma de todas as células de mamíferos (XIAO, 2004; AHN & AGGARWAL, 2005).

A família do NF-κB/Rel inclui NF-κB1 (p50/p105), NF-κB2 (p52/p100), p65 (RelA), RelB e c-Rel (CHEN *et al*., 1999) (Figura 9). Muitos membros desta família podem formar homodímeros ou heterodímeros. A forma ativada mais prevalente do NF-κB é o heterodímero formado pelas subunidades p50 ou p52 com a p65, que contêm domínios de transativação necessários para indução gênica (TAK E FARESTEIN, 2001).

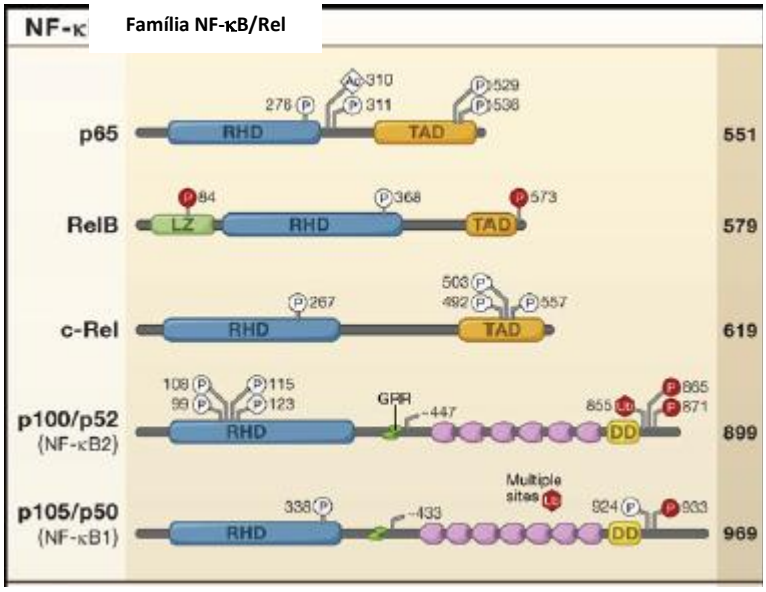

Figura 9. Membros da família do NF-κB (HAYDEN & GOSH, 2008).

Na sua forma inativa citoplasmática o NF-κB está associado com proteínas regulatórias chamadas de inibidoras de κB (IκB), onde as mais importantes são IκBα, IκBβ e IκBε. Além destas, existem a IκBζ (codificada pelo NFKBIZ), BCL-3 (linfoma 3 de células B) e a IκBNS (codificada pelo NFKBID) (Figura 10). Estas três últimas, geralmente não são expressas em células não estimuladas, mas são induzidas após ativação e medeiam seus efeitos no núcleo (HAYDEN & GHOSH, 2008). Por fim, um transcrito alternativo do gene *NFKB1* em camundongos codifica uma molécula de IκB, IκBγ, cujo papel biológico permanece obscuro. A IκBα, membro mais estudado, está associada com uma ativação transiente do NF-κB, enquanto que a IκBβ está envolvida com uma ativação sustentada (LI & NABEL, 1997).

A fosforilação da IκB, uma etapa importante na ativação do NF-κB, é mediada pelas IKB kinase (IKK). O complexo IKK consiste em pelo menos três subunidades, incluindo a IKK-α e IKK-β (também chamadas de IKK-1 e IKK-2, respectivamente) e a subunidade regulatória IKK-γ (também conhecida como NEMO) (YAMAOKA *et al*., 1998) (Figura 10). Uma forma induzível de IKK, conhecida como IKKi, foi identificada em células imunes estimuladas com endotoxinas (SHIMADA *et al*., 1999).

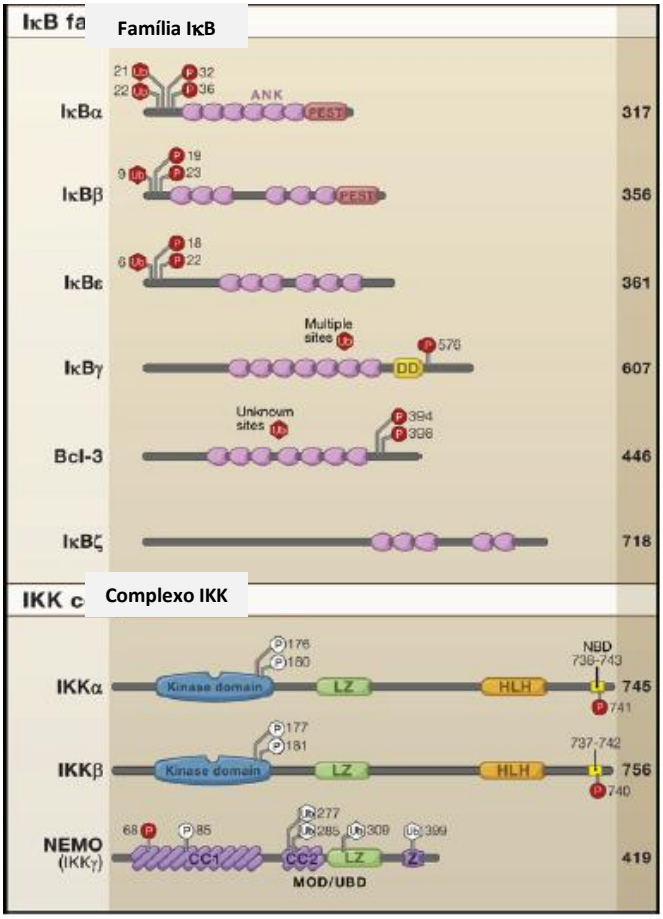

Figura 10. Família da IκB e complexo IKK (HAYDEN & GOSH, 2008).

Quando a célula é estimulada ocorre ativação da IKK que, promove a fosforilação das IkBs, causando sua degradação por proteossomos (DIDONATO *et al*., 1997). Isso resulta na liberação dos dímeros de NF-κB no citoplasma que, em seguida, se translocam para o núcleo para iniciar a expressão de genes-alvo (GHOSH & HAYDEN, 2008) (Figura 11).

As infecções bacterianas e virais (por exemplo, através do reconhecimento de produtos microbianos por receptores tais como os receptores Toll-like), citocinas inflamatórias e ligação de antígenos a receptores, podem levar à ativação do NF-κB, confirmando seu papel crucial nas respostas imune inata e adaptativa. Além disso, a ativação do NF-κB pode ser induzida por estresse físico (UV ou irradiação-γ), fisiológico (isquemia e choque hiperosmótico), ou oxidativo (BAEUERLE & HENKEL, 1994) (Figura 12).

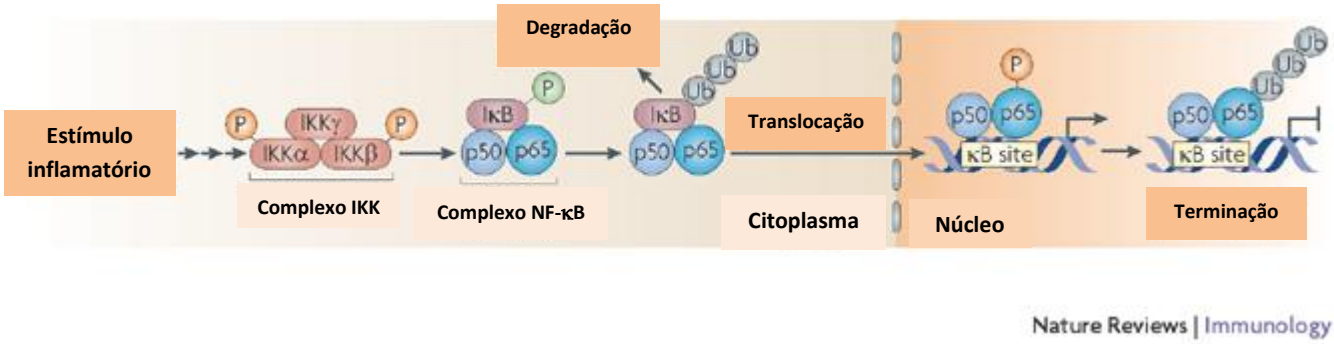

Figura 11. Via de ativação do NF-κB (GHOSH & HAYDEN, 2008).

O NF-κB regula a expressão de muitos genes envolvidos com as respostas imune e inflamatória atuando na ativação de genes de citocinas pro-inflamatórias, quimiocinas, enzimas que geram mediadores da inflamação, receptores imunológicos e moléculas de adesão, que apresentam papel no recrutamento inicial de leucócitos para o sítio de inflamação (BARNES & KARIN, 1997).

Os produtos dos genes que são regulados pelo NF-κB também causam sua ativação. Citocinas pro-inflamatórias como a IL-1β e o TNF-α, ativam e são ativadas pelo NF-κB. Esse tipo de regulação pode amplificar e perpetuar a resposta inflamatória local (BARNES & KARIN, 1997) (Figura 12).

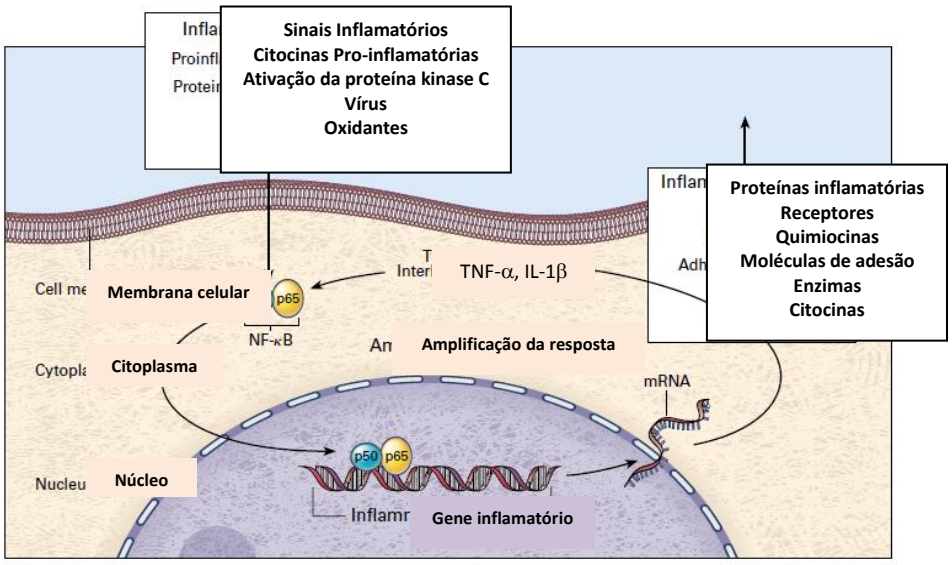

Figura 12. Diagrama esquemático do NF-κB como um regulador inflamatório (BARNES & KARIN, 1997).

O NF-κB também é capaz de atuar em conjunto com outros fatores de transcrição como o AP-1. Em sinoviócitos, essa duas vias aumentam a produção de citocinas pro-inflamatórias (TNF-α) e aumentam a expressão de enzimas destrutivas que regulam o remodelamento da matriz (YOKOO *et al*., 1996). O estímulo coordenado do NF-κB e do AP-1 pode contribuir para a destruição do osso e da cartilagem nas articulações. Na presença de acetaldeído, células HEPGE2 tiveram um aumento na ativação de NF-κB e AP-1 (ROMÁN *et al*., 2000). Esses resultados mostram-se relevantes para o estudo dos mecanismos envolvidos na DHA.

A identificação do NF-κB como um fator essencial na patogênese da inflamação tem desenvolvido estudos terapêuticos ligados a esse fator de transcrição para doenças humanas em modelos animais (TAK & FIRESTEIN, 2001). Uma variedade de fármacos usados no tratamento de doenças inflamatórias humanas tem efeito na atividade do NF-κB como antioxidantes, aspirina e corticosteroides (YAMAMOTO & GAYNOR, 2001). A identificação de componentes "chave" em uma doença é essencial para o desenvolvimento de uma terapêutica específica. Uma preocupação existente é com relação a toxicidade tecidual gerada pelo bloqueio da ativação do NF-κB. O benefício máximo dessa terapia dependerá do delicado equilíbrio entre a supressão da inflamação e manutenção normal da função celular. Com a seletividade de subunidades do NF-κB, de proteínas IκB ou kinases que tenham um certo grau de especificidade tecidual, pode-se atingir a eficácia terapêutica e minimizar a toxicidade sistêmica (TAK & FIRESTEIN, 2001).

4.6 TRATAMENTO DA DHA

Apesar da gravidade dessa doença, não existe uma terapia aprovada pelo comitê americano de Administração de Alimentos e Medicamentos (Food and Drug Administration - FDA). A abstinência alcóolica absoluta é essencial durante o tratamento de pacientes com DHA. Estes pacientes também devem parar de fumar e se forem obesos, perder peso. A obesidade e o cigarro estão associados ao estresse oxidativo e podem acelerar a progressão da doença por esta via. Muitos pacientes com

DHA apresentam desnutrição e um suporte nutricional é importante durante a terapia desses pacientes (MCCLAIN *et al*., 2004).

A farmacoterapia existente para DHA é bastante duvidosa. Desde que o estresse oxidativo foi implicado na patofisiologia, o uso de compostos naturais com propriedades antioxidantes tornou-se opção terapêutica mais popular no tratamento (BRUHA *et al*., 2012). O resveratrol é o antioxidante usado para prevenir o dano hepático por diminuir os radicais livres e citocinas inflamatórias em estudos experimentais (BISHAYEE *et al*., 2010).

A terapia com esteroides têm sido extensivamente estudada. Os corticosteroides representam a primeira forma de terapia medicamentosa em pacientes com DHA, onde o mecanismo de ação consiste na inibição da produção de citocinas pro-inflamatórias (MCCLAIN *et al*., 2004). A pentoxifilina é um inibidor de fosfodiesterase que bloqueia a transcrição de TNF-α que diminui os níveis séricos dos produtos desse gene e pode ser usada em pacientes com hepatite alcoólica severa (GAO & BATALLER, 2011). Medicamentos anti-TNF-α como o entanercept e o infliximab também são utilizados para o tratamento da DHA. Entretanto, mais estudos clínicos são necessários para padronizar ou combinar esses tratamentos porque esses medicamentos aumentam a mortalidade, o risco de infecções e a morte dos pacientes (BRUHA *et al*., 2012).

A identificação de alvos terapêuticos para a DHA tem sido dificuldade pelo fato de, na maioria dos modelos animais, a extensão da lesão hepática ser leve, e os animais não desenvolverem insuficiência hepática ou hipertensão portal grave. Modelos animais com as características da lesão hepática dos pacientes com a forma grave de DHA são necessários para que se possa avaliar os efeitos dos fatores envolvidos na patogênese. As amostras de fígado de pacientes com DHA são mais adequadas para identificar os alvos terapêuticos porque os níveis séricos de citocinas podem ser correlacionados com a severidade da doença. Mas, essas amostras têm menos significado fisiopatológico devido à depuração hepática e pela ocorrência de infecções bacterianas. Uma abordagem simples seria investigar a ativação ou expressão de diferentes mediadores do tecido hepático dos pacientes e relacioná-los com a gravidade da doença e em seguida testar a importância biológica desses fatores em modelos animais (GAO & BATALLER, 2011).

4.7 DIEILCARBAMAZINA

A dietilcarbamazina (DEC) é um derivado da piperazina sintetizada como 1-dietilcarbamil-4-metilpiperazina e preparada na forma de cloridrato, citrato ou fosfato (Figura 14). A partir de 1950, foi distribuída como sal citratado por inúmeras companhias farmacêuticas sob diferentes nomes, como Hetrazan, Banocide, Caricide, Carbilazine, entre outros. É um pó branco, muito solúvel em água, estável, mesmo em condições de umidade e temperatura muito elevadas, e resiste, inclusive, à autoclavagem. A denominação dietilcarbamazina genericamente se refere à sua forma citratada, uma vez que é mais comumente utilizada (DREYER & NORÕES, 1997).

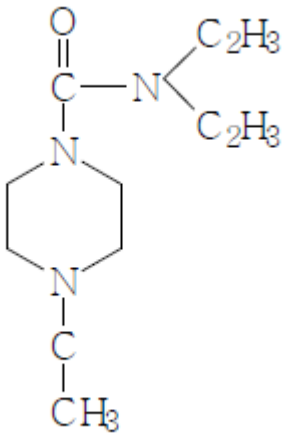

Figura 13. Citrato de dietilcarbamazina (Otssen, 1985).

A DEC é rapidamente absorvida pelo trato gastrointestinal e atinge o pico da sua concentração plasmática entre uma e três horas após a ingestão (HAWKING, 1979; 1977; SAKUMA *et al.*, 1967) e está quase ausente na urina, plasma e saliva de humanos após 24h da ingestão (ILONDU *et al.*, 2000). Por outro lado, estudos toxicológicos e farmacológicos em camundongos indicaram que após 3h o composto é completamente excretado pelo rim (HARNED *et al.*, 1948). Horii e Aoki (1997) descreveram o nível plasmático de DEC em ratos após a administração de 200 mg/kg, registrando valores de 30 µg/ml após 30-60 min da injeção, decrescendo rapidamente para 1,5 µg/ml após 4h e atingindo 0,1 µg/ml após 8h do tratamento.

Devido a sua ação microfilaricida eficaz (FLORENCIO & PEIXOTO, 2003) e macrofilaricida parcialmente eficazes (NORÕES *et al.*, 1997), a DEC é utilizada como tratamento padrão para filariose linfática desde meados de 1940. Inicialmente o regime de tratamento recomendado consistia de um curso de 12 dias de tratamento com DEC (6 mg/kg de peso). No entanto, pesquisas demonstraram que o tratamento com uma dose única de DEC (6 mg/kg de peso) tem eficácia microfilaricida a longo prazo comparável ao curso de 12 dias de tratamento (OTTENSEN, 2000).

Atualmente, têm-se realizado alguns estudos sobre o mecanismo de ação desta droga, que apesar de mais de 50 anos de uso, teve o seu potencial farmacológico tão pouco explorado. Sabe-se, até o momento, que parte dos efeitos atribuídos à DEC, deve-se a sua interferência no metabolismo do ácido araquidônico (NORÕES *et al.*, 1997).

Esta alteração do metabolismo do ácido araquidônico confere a DEC, propriedades anti-inflamatórias (MAIZELS & DENHAM, 1992). Sabe-se que a via do ácido araquidônico inclui as enzimas lipoxigenase (LOX) e ciclooxigenase (COX). A via da COX apresenta similaridade com a via do óxido nítrico, ambas possuem isoformas constitutivas e induzíveis de suas enzimas e controlam as respostas inflamatórias (CLANCY & ABRAMSON, 1995).

Existem poucos estudos sobre o papel da DEC na patofisiologia da inflamação. Alguns autores descreveram o uso terapêutico da DEC como droga anti-inflamatória para condições asmáticas (SALAZAR-MALLÉM, 1971; SRINIVAS & ANTANI, 1971; THIRUVENGADAM *et al.*, 1974; MAIZELS & DENHAM, 1992). O tratamento com DEC por 12 dias demonstrou um aumento no metabolismo do sulfactante pulmonar com uma posterior ativação de macrófagos alveolares, o que poderia explicar o alívio nos sintomas da asma, após o tratamento (FLORENCIO *et al.*, 2005). Segundo Queto *et al*. (2010), a DEC tem importante ação no bloqueio da inflamação eosinofílica pulmonar em camundongos sensibilizados com ovoalbumina. Foi observado que a DEC bloqueia a hiper-reatividade pulmonar, a produção de citocinas da resposta Th2 e o acúmulo de eosinófilos, bem como a eosinofilopoise *in vivo* e *in vitro*.

De acordo com Gonzalez et al. (1994), ratos com inflamação hepática induzida por tetracloreto de carbono (CCl_4) apresentaram uma evidente redução do dano morfológico após o tratamento com 25 e 50 mg/kg de DEC. Os animais apresentaram

organelas e sistema de membranas hepáticas bem preservados, mostrando o efeito protetor da DEC. Em outro estudo, Rocha et al. (2012) demonstraram que o tratamento com 50 mg/kg de DEC inibiu a injúria hepática e reduziu marcadores inflamatórios, como IL-6, TNF-α, MCP-1, iNOS durante a inflamação induzida pelo consumo crônico de etanol.

5 REFERÊNCIAS BIBLIOGRÁFICAS

ACHUR, R. N.; FREEMAN, W. M.; VRANA, K. E. Circulating cytokines as biomarkers of alcohol abuse and alcoholism. **J Neuroimmune Pharmacol**, 5: 83-91, 2010.

AGGARWAL, B. B. Nuclear factor-kappaB: the enemy within. **Cancer Cell**, 6: 203–208, 2004.

AHN, K.S.; AGGARWAL B. B. Transcription factor NF-kappaB: a sensor for smoke and stress signals. **Ann N Y Acad Sci**, 1056:218-33, 2005.

ALTAMIRANO, J.; BATALLER, R. Alcoholic liver disease: pathogenesis and new targets for therapy. **Gastroenterol**, 141(5):1572-85. 2011.

ARIAS-SALVATIERRA, D.; SILBERGELD, E. K.; ACOSTA-SAAVEDRA, L. C.; CALDERON-ARANDA, E. S. Role of nitric oxide produced by iNOS through NF-kappa B pathway in migration of cerebellar granule neurons induced by Lipopolysaccharide. **Cell Signal,** 23, 425–435, 2011.

ARMENDARIZ-BORUNDA, J.; SIMKEVICH, C. P.; ROY, N.; RAGHOW, R.; KANG, A. H.; SEYER, J. M. Activation of Ito cells involves regulation of AP-1 binding proteins and induction of type I collagen gene expression. **Biochem J**, 304: 817-824,1994.

BAEUERLE, P. A.; BALTIMORE, D. NF-kappa B: Ten years after. **Cell,** 87: 13–20, 1996.

BAEUERLE, P.A.; HENKEL, T. Function and activation of NF-k B in the immune system. **Annu Rev Immunol**, 12:141–179, 1994.

BALLAS, Z. K.; COOK, R. T.; SHEY, M. R.; COLEMAN, R. A. A Dynamic Flux in Natural Killer Cell Subsets as a Function of the Duration of Alcohol Ingestion. **Alcohol Clin Exp Res**, 36: 826-834, 2012.

BARNES, P. J.; KARIN, M. Nuclear factor-κB—a pivotal transcription factor in chronic inflammatory diseases. **New Eng J Med**,336: 1066-1071, 1997.

BAUMANN, H.; ONORATO, V.; GAULDIE, J.; JAHREIS, G.P. Distinct sets of acute phase plasma proteins are stimulated by separate human hepatocyte-stimulating factors and monokines in rat hepatoma cells. **J Biol Chem,** 262: 9756–9768, 1987.

BECKER, U.; DEIS, A.; SORENSEN, T.I.; et al. Prediction of risk of liver disease by alcohol intake, sex, and age: a prospective population study. **Hepatol**, 23:1025–9, 1996.

BEERS, M.H.; BERKOW, R. **Manual Merck: Diagnóstico e Tratamento – Edição Centenária**. 17. ed., São Paulo: Roca, 2001.

BELLENTANI, S.; SACCOCCIO, G.; COSTA, G.; et al. Drinking habits as cofactors of risk for alcohol induced liver damage. **The Dionysos Study Group**, 41:845–50. 1997.

BHASKARAN, N.; SHUKLA, S.; SRIVASTAVA, J.K.; GUPTA, S. Chamomile: an anti-inflammatory agent inhibits inducible nitric oxide synthase expression by blocking RelA/p65 activity. **Int J Mol Med,** 26: 935–940, 2010.

BISHAYEE A.; DARVESH A. S; POLITIS, T.; MCGORY, R. Resveratrol and liver disease: from bench to bedside and community. **Liver Int,** 30:1103-1114, 2010

BOELSTERLI, U.A.; BEDOUCHA, M. Toxicological consequences of altered peroxisome proliferator-activated receptor γ (PPARγ) expression in the liver: insights from models of obesity and type 2 diabetes. **Biochem Pharmacol,** 63:1–10, 2002.

BRUHA, R.; DVORAK, K.; PETRTYL, J. Alcoholic liver disease. **World J Hepatol,** 4: 81-90, 2012.

CHAE, H. B. Alcoholic liver disease. **Korean J Gastroenterol**, 53: 275-82. 2009.

CHARIYALERTSAK, S.; SIRIKULCHAYANONTA, V.; MAYER, D.; KOPP-SCHNEIDER, A.; FÜRSTENBERGER, G.; MARKS, F.; & MÜLLER-DECKER, K. Aberrant cyclooxygenase isozyme expression in human intrahepatic cholangiocarcinoma. **Gut,** 48: 80-86, 2001.

CHEN, F.; CASTRANOVA, V.; SHI, X.; DEMERS, L. M.. New insights into the role of nuclear factor-kappaB, a ubiquitous transcription factor in the initiation of diseases. **Clin Chem,** 45:7–17, 1999

CLANCY, R. M.; ABRAMSON, S. B. Nitric oxide: a novel mediator of inflammation. **Proc. Soc. Exp Biol Med**, 210: 93-10, 1995.

CRABB, D.W.; LIANGPUNSAKUL, S. Acetaldehyde generating enzyme systems: roles of alcohol dehydrogenase, CYP2E1 and catalase, and speculations on the role of other enzymes and processes. **Novartis Found Symp,** 285:4-16, 2007.

CRABB, D.W. Pathogenesis of alcoholic liver disease: newer mechanisms of injury. **Keio J Med,** 48:184-188, 1999.

CRAWFORD, J. M., The liver and biliary tract. In: Cotran, R.S., Kumar, V., Collins, T. **Pathologic Basis of Disease**, pp 831–896, 1994.

DAYNES, R. A.; JONES, D. C. Emerging roles of PPARs in inflammation and immunity. **Nat Rev Immunol,** 2:748–59, 2002.

DIDONATO, J.; MERCURIO, F.; ROSETTE, C.; et al. Mapping of the inducible I*k*B phosphorylation sites that signal its ubiquitination and degradation. **Mol Cell Biol,** 16:1295-304, 1996.

DING W.X.; LI, M, CHEN, X.; ET AL. Autophagy reduces acute ethanol induced hepatotoxicity and steatosis in mice. **Gastroenterol,** 139:1740–1752, 2010.

DINARELLO, C. A. Interleukin-1 and the pathogenesis of the acutephase response. **N Engl J Med.,** 311: 1413–1418, 1984.

DREYER, G.; NORÕES, J. Dietilcarbamazina no Tratamento da Filariose Bancroftiana. **Rev Soc Brasi Med Trop**, Brasília, v. 30, n. 3, p. 229- 240, 1997.

ENOMOTO, N.; TAKEI, Y.; HIROSE, M.; KONNO, A.; SHIBUYA, T.; MATSUYAMA, S., SUZUKI, S.; KITAMURA, K.I.; SATO, N. Prevention of ethanol-induced liver injury in rats by an agonist of peroxisome proliferator-activated receptor-gamma, pioglitazone. **J Pharmacol Exp Ther,** 306: 846-854, 2003.

FLORÊNCIO, M.S.; PEIXOTO, C.A. The effects of diethylcarbamazine on the ultrastructure of microfilarie of *Wuchereria bancrofti*. **Parasitol,** 126: 551-554, 2003.

FLORÊNCIO, M. S.; SARAIVA, K. L.; PEIXOTO, C. A. The effects of diethylcarbamazine on the ultrastructure of lung cells in vivo. **Tissue Cell** 37:241-6, 2005.

FUCHS, C. S.; STAMPFER, M. J.; COLDITZ, G. A.; et al. Alcohol consumption and mortality among women. **N Engl J Med,** 332: 1245–50, 1995.

FREEMAN, T. L.; TUMA, D. J.; THIELE, G. M.; KLASSEN, L.W.; WORRALL, S.; NIEMELA, O.; et al. Recent advances in alcohol-induced adduct formation. **Alcohol Clin Exp Res.,** 29:1310-6, 2005.

GALLI, A.; PINAIRE, J.; FISCHER, M.; DORRIS, R.; CRABB, D.W. The transcriptional and DNA binding activity of peroxisome proliferator-activated receptor alpha is inhibited by ethanol metabolism. A novel mechanism for the development of ethanol-induced fatty liver. **J Biol Chem,** 276: 68-75, 2001.

GAO, B.; BATALLER, R. Alcoholic liver disease: pathogenesis and new therapeutic targets. **Gastroenterol**, 141:1572-85, 2011.

GARTNER, L. P.; HIATT, J. L. **Tratato de Histologia em cores**. 3 ed., Rio de Janeiro: Saunders Elsevier; 2007.

GIANNITRAPANI, L.; INGRAO, S.; SORESI, M.; FLORENA, A. M.; SPADA, E. L.; SANDONATO, L.,D.; ALESSANDRO, N.; CERVELLO, M.; MONTALTO, G. Cyclooxygenase-2 Expression in Chronic Liver Diseases and Hepatocellular

Carcinoma. **Annals of the New York Academy of Sciences,** 1155: 293-299, 2009.

GONZALEZ, R.; ANCHETA, O.; MARQUEZ, M.; RODRIGUEZ, S. Hepatoprotective effects of diethylcarbamazine in acute liver damage induced by carbon tetrachloride in rats. **Acta Pharmacologica Sinica**, 15: 495-497, 1994

GHOSH, S.; HAYDEN, M. S. New regulators of NF-κB in inflammation. **Nat Rev Immunol,** *8*: 837-848, 2008.

HARDIE, D. G.; DAVID, C.; MARIAN, C. The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell?. **Ann Rev Biochem,** 67: 821-855,1998.

HARDIE, D. G. Regulation of fatty acid and cholesterol metabolism by the AMP-activated protein kinase. **Biochem Biophy Acta,** 1123: 231, 1992.

HARNED, B.K.; et al. Some toxicological and pharmacological properties of 1-diethylcarbamyl-4-methylpiperazine hydrochloride, hetrazan. **Annals of New York Academy of Science**, 141-160, 1948.

HAYDEN, M. S.; GHOSH, S. Shared principles in NF-κB signaling. **Cell,** 132: 344-362, 2008.

HAWKING, F. Diethylcarbamazine and new compounds for the treatment of filariasis. **Adv Pharmacol Chem**, 16:129-194, 1979.

Head, W. C. **Georgia DUI Alcohol Metabolism Rate (2011)**. Disponível em: <http://www.absolutely-not-guilty.com/georgiaduialcoholmetabolismrate.html>. Acesso: 20/06/2012.

HELMAN, R. A.; TEMKO, M. H.; NYE, S. W.; et al. Alcoholic hepatitis. Natural history and evaluation of prednisolone therapy. **Ann Intern Med**, 74:311–321, 1971.

HOEK, J. B.; PASTORINO, J. G. Cellular signaling mechanisms in alcohol-induced liver damage. **Semin Liver Dis.**, 24:257–72, 2004.

HOLFORD, G.H. Clinical pharmacokinetics of ethanol. **Clin Pharmacokinetics**, 13:273-292, 1987.

HORII, Y.; AOKI, Y. Plasma levels of diethylcarbamazine and their effects on implanted microfilariae of Brugia pahangi in rats. **J Vet Med Scien**, 59: 961-963, 1997.

HORTON, J. D.; GOLDSTEIN, J.L.; BROWN, M. S. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. **J Clin Invest**, 109: 1125-1131, 2002.

ILONDU, N.; ORISAKWE, O. E.; OFOEFULE, S. I.; AFONNE O. J.; OBI, E.; CHILAKA, K. C.; ORISH, C. Pharmacokinetics of diethylcarbamazine: Prediction by concentration in saliva. **Biol Pharm Bull**, 23:443-445, 2000.

ISSELBACHER, K. J.; PODOLSKY, D. K. Biologic and clinical approaches to liver disease. In: Wilson, J.D., Braunwald, E., Isselbacher, K.J., Petersdorf, R.G., Martin, J.B., Fauci, A.S., Root, R .K. (Eds.), **Harrison's Principles of Internal Medicine.** McGraw-Hill, Inc., New York, pp. 1301–1302, 1991.

JUQUEIRA, L. C.; CARNEIRO, J. **Histologia Básica**. 11 ed., Rio de Janeiro: Guanabara Koogan; p.524, 2008.

KARINCH, A. M.; MARTIN, J. H.; VARY, T. C. Acute and chronic ethanol consumption differentially impact pathways limiting hepatic protein synthesis. **Am J Physiol Gastrointest Liver Physiol,** 295*:* E3-E9, 2008.

KISHORE, R.; HILL, J. R.; MCMULLEN, M. R.; FRENKEL, J.; NAGY, L. E. ERK1/2 and Egr-1 contribute to increased TNF-alpha production in rat Kupffer cells after chronic ethanol feeding. **Am J Physiol Gastrointest Liver Physiol**, 282:G6–G15, 2002.

KANG, X.; ZHONG, W.; LIU, J.; et al. Zinc supplementation reverses alcohol-induced steatosis in mice through reactivating hepatocyte nuclear factor-4alpha and

peroxisome proliferator-activated receptor-alpha. **Hepatology,** 50:1241–1250, 2009.

KUNITOH, S.; IMAOKA, S.; HIROI, T.; YABUSAKI, Y.; MONNA, T.; FUNAE, Y. Acetaldehyde as well as ethanol is metabolized by human CYP2E1. **J Pharmacol Exp Ther**., 280:527-32, 1997.

LI, Z.; NABEL, G. J. A new member of the I kappaB protein family, I kappaB epsilon, inhibits RelA (p65)-mediated NF-kappaB transcription. **Mol Cell Biol**, 17:6184–6190, 1997.

LIEBER, C.S.. Metabolism of Alcohol. **Clin Liver Dis**, 9: 1-35, 2005.

LIEBER, C. S. Alcohol and the liver: metabolism of alcohol and its role in hepatic and extrahepatic diseases. **Mt Sinai J Med**, 67: 84, 2000.

LIEBER, C.S. Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. **Alcohol,** 34:9e19. 2004.

LU, Y.; ZHUGE, J.; WANG, X.; et al. Cytochrome P450 2E1 contributes to ethanol-induced fatty liver in mice. **Hepatology,** 47: 1483–1494,2008.

MAIZELS, R. M.; DENHAM, D. A. Diethilcarbamazine (DEC): immunopharmacological interactions of an anti-filarial drug. **Parasitology**, 105: 849-860, 1992.

MAHTANI, K.R.; BROOK, M.; DEAN, J. L.; SULLY, G.; SAKLATVALA, J.; CLARK, A. R. Mitogen-activated protein kinase p38 controls the expression and posttranslational modification of tristetraprolin, a regulator of tumor necrosis factor alpha mRNA stability. **Mol Cell Biol,** 21: 6461-6469,2001.

MANDAYAM, S.; JAMAL, M. M.; MORGAN, T. R. Epidemiology of alcoholic liver disease. **Semin Liver Dis,** 24: 217–32, 2004.

MANDREKAR, P. Signaling mechanisms in alcoholic liver injury: Role of transcription factors, kinases and heat shock proteins. **World J Gastroenterol,** 13: 4979-4985, 2007.

MANDREKAR, P.; SZABO, G. Signalling pathways in alcohol-induced liver inflammation. **J Hepatol,** 50: 1258-1266, 2009.

MATSUI, K.; OZAKI, T.; OISHI, M.; TANAKA, Y.; KAIBORI, M.; NISHIZAWA, M.; et al. Active hexose correlated compound inhibits the expression of proinflammatory biomarker iNOS in hepatocytes. **Europ Surg Res**, 47*:* 274-283, 2011.

MCCLAIN, C. J.; SONG, Z.; BARVE, S. S.; HILL, D. B.; DEACIUC, I. Recent advances in alcoholic liver disease IV. Dysregulated cytokine metabolism in alcoholic liver disease. **Am J Physiol Gastrointest Liver Physiol**, 287, G497-G502, 2004.

MCCLAIN, C.J.; HILL, D.B.; SONG, Z., DEACIUC, I., BARVE, S. Monocyte activation in alcoholic liver disease. **Alcohol,** 27: 53-61, 2002.

MCMULLEN, M. R.; PRITCHARD, M.T.; WANG, Q.; MILLWARD, C.A.; CRONIGER, C.M.; NAGY, L.E. Early growth response-1 transcription factor is essential for ethanol-induced fatty liver injury in mice. **Gastroenterol,** 128: 2066-2076, 2005.

MEMON, R.A.; TECOTT, L. H.; NONOGAKI, K.; BEIGNEUX, A.; MOSER, A. H.; et al. Up-regulation of peroxiso me proliferatoractivated receptors (PPAR-α) and PPAR-γ messenger ribonucleic acid expression in the liver in murine obesity: troglitazone induces expression of PPAR-γ -responsive adipose tissue-specific genes in the liver of obese diabetic mice. **Endocrinology**, 141:4021–31, 2000.

MICHALIK, L.; JOHAN, A.; JOEL, P. B.; KRISHNA, C.; CHRISTOPHER, K. G.; FRANK, J. G.; PAUL, A. G.; et al. Peroxisome proliferator-activated receptors. **Pharmacol Rev,** 58:726-741, 2006.

NANJI, A. A.; JOKELAINEN, K.; RAHEMTULLA, A.; MIAO, L.; FOGT, F.; MATSUMOTO, H.; et al. Activation of nuclear factor kappa B and cytokine imbalance in experimental alcoholic liver disease in the rat. **Hepatology,** 30: 934-943,1999.

NIEMELA, O. Acetaldehyde adducts in circulation. **Novartis Found Symp,** 285:183-92; discussion 93-7, 2007.

NORÕES, J.; DREYER, G.; SANTOS, A.; MENDES, V. G.; MEDEIROS, Z.; ADDISS, D. Assessment of the efficary of diethylcarbamazine on adult *Wuchereria bancrofti*. **Trans R Soc Trop Med Hyg**, 91: 78-81, 1997.

OTTESEN, E.A. Efficacy of diethylcarbamazine in eradicating infection with lymphaticdwelling filarial in humans. **Rev Infec Dis**, 7: 341-356, 1985.

OTTESEN, E.A.; HOOPER, P.J.; BRADLEY, M.; BISWAS, G. The Global Programme to Eliminate Lymphatic Filariasis: health impact after 8 years. **PLoS Negl Trop Dis.,** 2:e317, 2008.

POMPOSELLI, J.J.; FLORES, E. A.; BISTRIAN, B.R. Role of biochemical mediators in clinical nutrition and surgical metabolism. JPEN J. **Parenter Enteral Nutr,** 12: 212–218, 1988.

QUETO, T.; XAVIER-ELSAS, P.; GARDEL, M. A.; DE LUCA, B.; BARRADAS, M.; MASID, D.; E SILVA, P. M.; PEIXOTO, C. A.; VASCONCELOS, Z. M.; DIAS, E. P.; GASPAR-ELSAS, M. I. Inducible nitric oxide synthase/CD95L-dependent suppression of pulmonary and bone marrow eosinophilia by diethylcarbamazine. **Am J Resp Crit Care Med**, 181:429-37, 2010.

RAHIMIAN, R.; MASIH-KHAN, E.; LO, M.; VAN BREEMEN, C.; MCMANUS, B. M.; DUBE, G. P. Hepatic over-expression of peroxisome proliferator activated receptor γ 2 in the ob/ob mouse model of non-insulin dependent diabetes mellitus. **Mol Cell Biochem**, 224:29–37, 2001.

ROCHA, S. W. S.; SILVA, B. S.; GOMES, F. O. S.; SILVA, A. K. S.; RAPOSO, C.; BARBOSA, K. P. S.; TORRES, D. O. C.; SANTOS, AC. O.; PEIXOTO, C. A.

Effect of Diethylcarbamazine on chronic hepatic inflammation induced by alcohol in C57BL/6 mice. **Eur J Pharmacol**, 689: 194-203, 2012.

ROMÁN, J.; GIMENÉZ, A.; LLUIS, J. M.; GASSÓ, M.; RUBIO, M.; CABALLERIA, J.; PARÉS, A.; RODÉS, J.; FERNÁNDEZ-CHECA, J. C. Enhanced DNA Binding and Activation of Transcription Factors NF-kB and AP-1 by Acetaldehyde in HEPG2 Cells. **J Biol Chem,** 275: 14684–14690, 2000.

ROUAULT, T. A. Hepatic iron overload in alcoholic liver disease: why does it occur and what is its role in pathogenesis? **Alcohol,** 30: 103–6, 2003.

SAFDAR, K.; SCHIFF, E. R. Alcohol and hepatitis C. **Semin Liver Dis.,** 24: 305–15, 2004.

SAKUMA, S.; et al. Study of the distribution of the antifilarial agent diethylcarbamazine within the body using tritium labeling and whole-body autoradiography. **Japan J Parasitol**, 16:179-187, 1967.

SALAZAR-MALLÉN, M. Treatment of intractable asthma with diethylcarbamazine citrate. **Ann Allergy**, 23: 534-537, 1971.

SERRANO-MARCO, L.; CHACÓN, M. R.; MAYMÓ-MASIP, E.; BARROSO, E.; SALVADÓ, L.; WABISTCH, M.,; et al. TNF-α inhibits PPARβ/δ activity and SIRT1 expression through NF-κB in human adipocytes. **Biochim et Biophy Acta,** 1821:1177-85, 2012.

SHAULIAN, E.; KARIN, M. AP-1 as a regulator of cell life and death. **Nat Cell Biol,** 4: E131-E136, 2002

SHERLOCK, S.; DOOLEY, J. Drugs and the liver. **Dis liv biliar system**, 10: 337-69, 2002.

SHIMADA,T.; et al. IKK-i, a novel lipopolysaccharide-inducible kinase that is related to IkappaB kinases. **Int Immunol**, 11:1357–1362, 1999.

SORENSEN, T. I.; ORHOLM, M.; BENTSEN, K. D.; HOYBYE, G.; EGHOJE, K.; CHRISTOFFERSEN, P. Prospective evaluation of alcohol abuse and alcoholic

liver injury in men as predictors of development of cirrhosis. **Lancet,** 2: 241–4, 1984.

STEWART, S.; JONES, D.; DAY, C. P. Alcoholic liver disease: new insights into mechanisms and preventative strategies. **Trends Mol Med,** 7: 408–13, 2001.

SWEET, M. J.; HUME, D. A. Endotoxin signal transduction in macrophages. **J Leukoc Biol**, 60: 8-26, 1996.

TAK, P. P.; FIRESTEIN, G. S. NF-κB: a key role in inflammatory diseases. **J Clin Inves,** 107, 7-11, 2001.

TELI, M. R.; DAY, C. P.; BURT, A. D. Determinants of progression to cirrhosis or fibrosis in pure alcoholic fatty liver. **Lancet** 346:1562–3, 1995.

THIRUVENGADAM, K. V. et al. Diethylcarbamazine citrate in bronchial asthma. **J Indian Med Assoc**,63: 278-281, 1974.

THUN, M.J.; PETO, R.; LOPEZ, A.D.; et al. Alcohol consumption and mortality among middle-aged and elderly US adults. **N Engl J Med** 337: 1705–14. 1997.

TILG, H.; DIEHL, A.M. Cytokines in alcoholic and nonalcoholic steatohepatitis. **N Engl J Med** 343: 1467–76, 2000.

TOME, S.; LUCEY, M. R. Review article: current management of alcoholic liver disease. **Aliment Pharmacol Ther,** 19: 707–14, 2004.

TUMA, D. J.; CASEY, C. A. Dangerous byproducts of alcohol breakdown--focus on adducts. **Alcohol Res Health,** 27:285-90, 2003.

VIDAL-PUIG, A., JIMENEZ-LINAN, M.; LOWELL, B. B.; HAMANN, A.; HU, E.; et al. Regulation of PPARγ gene expression by nutrition and obesity in rodents. **J Clin Invest**, 97:2553–61, 1996.

VIOLLET, B.; GUIGAS, B.; LECLERC, J.; et al. AMP-activated protein kinase in the regulation of hepatic energy metabolism: from physiology to therapeutic perspectives. **Acta Physiol,** 196:81–98, 2009.

WAGNER, M.; ZOLLNER, G.; TRAUNER, M. Nuclear receptors in liver disease. **Hepatology**, 53:1023–1034, 2011.

WANG, Y.; MILLONIG, G.; NAIR, J.; et al. Ethanol-induced cytochrome P4502E1 causes carcinogenic etheno-DNA lesions in alcoholic liver disease. **Hepatology,** 50:453 -61, 2009.

WANLESS, I. R., Physioanatomic considerations. In: Schiff, E.R., Sorrell, M.F., Maddrey, W.C. (Eds.), **Schiff's Diseases of the Liver**. Lippincott-Raven, Philadelphia, pp. 3–38, 1999.

WEATHERMON, R.; CRABB, D. W. Alcohol and medication interactions. **Alcohol Res Health,** 23:40-54, 1999.

WHEELER, M. D.; THURMAN, R. G. Upregulation of CD14 in liver due to acute ethanol involves oxidant-dependent AP-1 pathway. **J Biol Chem**, 278:8345–8351, 2003.

WORLD HEALTH ORGANIZATION. 2011. Global Status Report on Alcohol and Health. Geneva: WHO Press.

WU, D.; CEDERBAUM, A. I. Oxidative stress and alcoholic liver disease. **Semin Liver Dis,** 29:141-54, 2009.

XIAO, W. Advances in NF-κB signaling transduction and transcription. **Cell Mol Immunol**, 1:425–433, 2004.

YAN, S. F.; FUJITA, T.; LU, J.; OKAD, AK.; ZOU, Y. S.; MACKMAN, N.; et al. Egr-1, a master switch coordinating upregulation of divergent gene families underlying ischemic stress. **Nat Med**, 6:1355–1361, 2000.

YAMAMOTO, Y.; GAYNOR, R. B. Therapeutic potential of inhibition of the NF-kB pathway in the treatment of inflammation and cancer. **J Clin Invest**, In press. 2001.

YAMAOKA, S., et al. Complementation cloning of NEMO, a component of the IkappaB kinase complex essential for NF-kappaB activation. **Cell**, 93:1231–1240, 1998.

YIN, M.; WHEELER, M. D.; KONO, H.; BRADFORD, B. U.; GALLUCCI, R. M.; LUSTER, M.I.; THURMAN, R. G. Essential role of tumor necrosis factor alpha in alcohol-induced liver injury in mice. **Gastroenterology**, 117:942-952, 1999.

YOKOO, T.; KITAMURA, M. Dual regulation of IL-1 beta-mediated matrix metalloproteinase-9 expression in mesangial cells by NF-kappa B and AP-1. **Am J Physiol**, 270:F123–F130, 1996.

YOU, M.; FISCHER, M.; DEEG, M.A.; et al. Ethanol induces fatty acid synthesis pathways by activation of sterol regulatory elementbinding protein (SREBP). **J Biol Chem**, 277:29342–29347, 2002.

YOU, M.; MATSUMOTO, M.; PACOLD, C. M.; et al. The role of AMP-activated protein kinase in the action of ethanol in the liver. **Gastroenterology**, 127:1798–1808, 2004.

YOU, M.; CONSIDINE, R. V.; LEONE, T.C.; et al. Role of adiponectin in the protective action of dietary saturated fat against alcoholic fatty liver in mice. **Hepatology,** 42:568–577, 2005.

YU, S., RAO, S.; REDDY, J.K. Peroxisome proliferator-activated receptors, fatty acid oxidation, steatohepatitis and hepatocarcinogenesis. **Curr Mol Med,** 3:561–572, 2003.

ZAKHARI, S. Overview: how is alcohol metabolized by the body?. **Alcohol Res Health**. 29:245-54, 2006.

CAPÍTULO II

Diethylcarbamazine prevents alcohol-induced liver injury in C57BL/6 mice by inhibiting the NF-κB activation

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Abstract

Induction of NF-κB-mediated gene expression has been identified in the pathogenesis of alcoholic liver disease (ALD). Diethylcarbamazine (DEC) is a piperazine derivative drug with anti-inflammatory properties. The present study was designed to evaluate the effect of DEC on NF-κB pathways in mice undergoing alcoholism induced hepatic inflammation. Forty male C57BL/6 mice were divided equally into four groups: control group (C); DEC- treated group, which received 50 mg/kg (DEC50); alcoholic group (EtOH), submitted to chronic alcohol consumption and alcohol-DEC treated group (EtOH50), submitted to chronic alcoholism consumption plus DEC treatment. Histological analysis of the alcoholic group showed evident hepatocellular damage which was reduced in the EtOH50 group. Immunohistochemistry and western blot results showed elevated expression of inflammatory markers such as MDA, TNF-α, IL-1β, COX-2 and iNOS in hepatocytes of EtOH group. However, low immunopositivity for these markers was detected following DEC treatment. In the EtOH group the activation of NF-κB was observed by an increase in the expression of both NF-κB and pNF-κB in hepatocytes. This expression was significantly reduced in livers of EtOH50 group. Protein expression of Iκβα was measured to determine whether activation of NF-κB might be the result of Iκβα degradation. It was observed that expression of this enzyme was low in EtOH group, while animals treated with DEC had a high expression of Iκβα. The results of the present study indicate that DEC alleviates alcoholic liver injury, in part by the inhibiting activation of NF-κB and by suppressing the induction of NF-κB-dependent genes.

Keywords: Diethylcarbamazine, Alcoholism, Hepatic injury, Inflammatory markers, transcription factors, NF-κB

Abbreviations: ALD, alcoholic liver disease; DEC, diethylcarbamazine; MDA, malondialdehyde; TNF-α, tumor necrosis factor-α; IL-1β, interleukin 1-β; COX-2, cyclooxygenase-2; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; NF-κB, nuclear factor-kappa B; WB, Western blot; NO nitric oxide; TXB, thromboxanes; TGFb, transforming growth factor-β.

1. Introduction

The hazardous and harmful use of alcohol is a major contributing global factor to death, disease and injury. Alcohol consumption is directly associated with liver disease mortality and leads to elevated social and economic costs (Bruha et al., 2012). The most commonly recognized symptoms of alcohol consumption are associated with chronic alcoholism, and it is a causal or risk factor in 60 types of diseases and injuries. These and other effects of alcohol consumption have made alcohol the third leading global risk factor for disease and disability (WHO, 2011).

The toxic effects of alcohol have an impact on multiple organs; however, the liver, as the primary site of alcohol metabolism, is the major injury target (Karinch et al, 2008; Lieber, 2000; 2005). Increasingly, alcoholic liver disease (ALD) is considered to be a complex and multifaceted pathological process, involving oxidative stress, inflammation and excessive fatty acid synthesis (Mandrekar, 2007). The progression of the disease involves various pro-inflammatory molecules such as interleukins, cytokines, adhesion molecules and nuclear factor-kappa B (NF-κB) (Achur et al., 2010, Ballas et al., 2012, Rocha et al., 2012).

NF-κB is a transcription factor involved in inflammation and immune response (Baeuerle and Baltimore, 1996) and is activated by oxidants and cytokines such as interleukin 1β (IL-1β) and cytokine tumor necrosis factor-alpha (TNF-α) (Barnes and

Karin, 1997), which play important roles in inflammation and the development of ALD. IL-1β and TNF-α trigger the degradation and phosphorylation of IκB proteins, thus permitting the entry of NF-κB p65/p50 into the nucleus, where it activates the transcription of various genes including cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), both considered important mediators in the recruitment of inflammatory cells (Bhaskaran et al., 2010; Arias-Salvatierra et al., 2011).

Since 1947 diethylcarbamazine (DEC) is the most widely used drug for the treatment of lymphatic filariasis (Hewitt et al., 1947). As a result of its interference with arachidonic acid metabolism, DEC has anti-inflammatory properties, including lipoxygenase (LOX) and cyclooxygenase (COX) enzymes (Maizels and Denham, 1992; McGarry et al., 2005). Queto et al. (2010) demonstrated that DEC has important role in blocking pulmonary eosinophilic inflammation in mice sensitized with ovalbumin, effectively preventing the effects of subsequent airway resistance, Th1/Th2 cytokine production, pulmonary eosinophil accumulation and *in vivo* and *ex vivo* eosinophilopoiesis. Additionally, DEC directly suppressed IL-5–dependent eosinophilopoiesis in naive bone marrow.

Recently, Rocha et al. (2012) demonstrated that chronic consumption of ethanol increases NF-κB levels and targets several proinflammatory cytokines, chemokines and oxidases. The administration of DEC inhibits hepatic injury and decreases inflammatory markers, suggesting potential therapeutical use in chronic inflammation induced by alcoholism. However, the inhibition of the nuclear transcription factors of the κB family pathways needs to be clarified. Therefore, the aim of the present study was to investigate the role of DEC on NF-κB pathways in hepatic inflammation induced by alcoholism. The following points were examined: 1) hepatic injury (histology), 2)

detection of TNF-α, IL-1β, malondialdehyde (MDA), COX-2 and NF-κB (immunohistochemistry), 3) expression of IL-1β, COX-2, TNF-α, IκB and NF-κB (western blot).

2. Material and Methods

2.1 Animals

Forty male C57BL/6 mice aged 5 weeks and weighting 15-17 g were used in all experiments. The health of the mice was examined and they were acclimatized to the laboratory environment of 22 ^{o}C and 12 h light: 12 dark photoperiods. The animals were housed in metal cages and fed a standard diet and water *ad libitum*. The animal studies Ethics Committee of Oswaldo Cruz Institute approved all the experiments reported (CEUA LW- 32/10).

2.2 Diethylcarbamazine solutions

The solutions were compounds of distilled water and DEC (Farmanguinhos, FIOCRUZ, Brazil) adjusted according to the body weight of each animal. The treated groups received 50mg/kg of DEC for twelve days through gavage (200 μl) (Rocha et al., 2012; Saraiva et al., 2006). The control group received distilled water only, administered in the same way. Classical metabolic studies describe the total metabolic rate of a 30 g mouse as 961 kJ per kg body weight, which is approximately seven times the total metabolic rate of a 70 kg human (138 kJ per kg (Terpstra, 2001)). The lymphatic filariasis therapeutic dose regimens recommended by the World Health Organization (WHO) is 6mg/kg for 12 days (WHO, 2011). In the present study, 50 mg/kg of DEC for 12 days was used, which is approximately seven times the human dose.

2.3 Experimental groups

After a week of acclimation, 30 day old C57BL/6 mice were separated into four groups (n=10 each): control group (C) that received distilled water only, administered in the same way, DEC-treated group (DEC50) that received 50 mg/kg DEC for twelve days by gavage, alcoholic group (EtOH) which received ethanol and alcoholic plus 50 mg/kg DEC group (EtOH50). Ethanol was provided in drinking water at 10% (v/v) for 2 days, 15% for 5 days, and 20% for 5 weeks (Ballas et al., 2012; Cook et al., 2004). According to Cook et al. (2004), innate immune system of mice presents significant changes after 20% ethanol consumption for three weeks. Solutions were changed daily and the remaining liquid in the bottles was measured in a graduated cylinder for final volume analysis, and then discarded. After five weeks of alcoholism induction, mice received alcohol plus treatment for 12 days with a solution of DEC administered orally. The alcoholic group received alcohol plus water by gavage for 12 days.

2.4 Histopathology

Liver fragments were fixed in 10% formalin for 24 hours, processed and embedded in paraffin. Sections of 4-5µm were cut and mounted on glass slides. The sections were stained with hematoxylin-eosin (HE) and evaluated with an inverted microscope (Observer Z1, Zeiss MicroImaging GmbH) equipped with a camera and 4.7.4 image analysis program (AxionCam MRm Zeiss) at a magnification of 400 x.

2.5 Immunohistochemical assays

Five sections (5 µm in thickness) of each group were cut and adhered to slides treated with 3-amino-propyl-trietoxi-silane (APES [Sigma, USA]). Briefly, the sections were deparaffinized with xylene and rehydrated in graded ethanol (100 to 70%). To increase epitope exposure, the sections were heated for 30 minutes in a sodium citrate buffer (0.01 M, pH 6.0). To minimize endogenous peroxidase activity, the slides were treated with 0.3% (v/v) H_2O_2 in water for five minutes. The sections were washed with

0.01M PBS (pH 7.2) and then blocked with 1% BSA, 0.2% Tween 20 in PBS for 1h at room temperature. The sections were then incubated for 12 hours at 4ºC with pantibody against NF-κB-p65 (1:50, Santa Cruz Biotechnology, CA), pNF-κB (1:100, Abcam, Cambridge, UK), IκBα (1:100, Santa Cruz Biotechnology, CA), TNF-α (1:50, Abcam, Cambridge, UK), MDA (1:50, Abcam Cambridge, UK), IL-1β (1:250, Abcam, Cambridge, UK), COX-2 (1:400; Abcam, Cambridge, UK), iNOS (1:50 Abcam, Cambridge, UK). The antigen-antibody reaction was visualized with avidin-biotin peroxidase (Dako Universal LSAB ® + Kit, Peroxidase) using 3.3-diaminobenzidine as the chromogen. The slides were counterstained with hematoxylin. Positive staining resulted in a brown reaction product. Negative controls were treated as above, but with the omission of the first antibody. Five pictures at the same magnification were quantitatively analyzed using Gimp 2.6 software (GNU Image Manipulation Program, UNIX platforms).

2.6 Total protein extraction

Livers were quickly dissected and then homogenized in a Wheaton Overhead Stirrer (No 903475) in an extraction cocktail (10 mM Ethylenediamine tetraacetic acid (EDTA), 2 mM phenylmethylsulfonyl fluoride (PMSF), 100 mM sodium fluoride, 10mM sodium pyrophosphate, 10 mM sodium orthovanadate ($NaVO_4$), 10 mg of aprotinin and 100 mM Tris(hydroxymethyl)aminomethane, pH 7.4). Homogenates were centrifuged at 3000 xg for 10 min and the supernatant was collected and stored at -80º C until use for IL-1B and COX-2 immunoblotting.

2.7 Cytosolic and Nuclear protein extraction

Cytosolic and the nuclear proteins of liver were isolated using Cayman's Nuclear Extraction kit (Item No. 10009277, Cayman chemical company, Ann Arbor, Michigan, USA). Liver fragments were briefly homogenized in a hypotonic buffer

supplemented with DTT and Nonidet P-40 per gram of tissue. The livers were centrifuged and re-suspended by adding specified assay reagents as instructed. The cytosolic and nuclear fractions were stored in pre-chilled vials at -80˚C until further analysis. Livers cytosols were used to determine amounts of IκBα in immunoblotting, and nuclear fractions were used for NF-κB immunoblotting.

2.8 Measurement of protein levels

Total, cytosolic and nuclear extraction protein levels were determined through the Bradford method using bovine serum albumin as standard (Bradford, 1970). The samples were read in a spectrophotometer at 660nm. All samples were run in duplicates and an average of the two absorbency levels was used to determine protein quantity. The protein concentration per sample amount was determined using the equation from a calibration curve. The curve was generated using the same method as the samples, with the substitution of bovine serum albumin at five concentration levels.

2.9 Western blot

The proteins (40 μg) were separated on 10% (NF-κB, pNF-κB, IκBα, pIκBα and COX-2) or 12% (IL-1β) sodium dodecyl sulfate–polyacrylamide by gel electrophoresis under reduced conditions and were electrophoretically transferred onto nitrocellulose membrane (Bio Rad, CA, USA, Ref. 162-0115). After overnight blocking at 4ºC with 5% non-fat milk in TBS-T (Tris-buffered saline 0.1% plus 0.05% Tween 20, pH 7.4), the membranes were incubated at room temperature for 3h with antibody against NF-kB (1:200, Santa Cruz Biotechnology, CA), pNF-kB (1:200, Abcam Cambridge, UK) IkBα (1:500, Santa Cruz Biotechnology, CA), p IkBα (1:50, Cell Signaling Technology, USA), IL-1β (1:1000, Abcam Cambridge, UK) and COX-2 (1:1000, Abcam Cambridge, UK), diluted in TBS-T buffer solution containing 3% non-fat milk. After washing (six times, 10 min each) in TBS-T, the membranes were further

reacted with horseradish peroxidase-conjugated anti-rabbit antibody (1:80000, Sigma, USA), diluted in TBS-T with 1% nonfat milk, for 1h30min, at room temperature. An enhanced chemiluminescence reagent (Super Signal, Pierce, Ref. 34080) was used to make the labeled protein bands visible and the blots were developed on X-ray film (Fuji Medical, Kodak, Ref. Z358487-50EA). For quantification, the density of pixels of each band was determined by the Image J 1.38 program (available at http://rsbweb.nih.gov/ij/download.html; developed by Wayne Rasband, NIH, Bethesda, MD). For each protein investigated the results were confirmed in three sets of experiments. Immunoblot for β-actin was performed as a control for the above protein blots. After protein blot visualization with enhanced chemiluminescence, the protein antibodies were stripped from the membranes, which were reprobed with monoclonal anti-β-actin antibody (1:2000, Sigma, USA), and protein densitometry was performed.

2.10 Statistical analysis

For statistical analysis GraphPad Prism software, version 5, was used. Results were expressed with mean ± standard deviation. Differences between control and treated groups were analyzed using analysis of variance T tests as *post hoc* tests. Probability values less than 0.05 were considered significant.

3. Results

3.1 Ethanol consumption and Body mass

No significant difference in ethanol consumption was observed during the experiment between the alcoholic group EtOH (171.5 ±6.543) and EtOH50 (173.5 ± 5.525). Additionally, no difference was observed in body mass between the EtOH (24.61 ± 0.4610) group and the EtOH50 group (24.33 ± 0.4248).

3.2 DEC inhibits alcohol-induced liver injury

No histological alteration was observed in the livers of mice from the control group (C) or 12 days of DEC treatment (DEC50) group. Mice from these groups had well-preserved tissue, composed of radially arranged cords of hepatocytes distributed in hepatic lobules (Fig. 1A and 1B).

In the livers of the group that received chronic ethanol for 5 weeks (EtOH) the presence of some areas of interstitial edema parallel to the cords of hepatocytes and lipid droplets in the cytoplasm of hepatocytes were observed, characteristic of the steatosis process. Moreover, necrosis and inflammation was observed in the liver parenchyma (1C). The group that received ethanol and was treated for 12 days with 50 mg/kg of DEC (EtOH50) had a reduced number of lipid droplets and a decrease in inflammation and necrosis (Fig. 1D).

3.3 DEC treatment inhibits alcohol-induced lipid peroxidation in liver

Malondialdehyde (MDA) is a product of lipid peroxidation that effects the modulation of hepatic inflammatory response. Administration of alcohol produces severe liver damage, as indicated by markedly increased MDA levels. Immunohistochemical staining showed that MDA was highly accumulated in the hepatic tissue of the EtOH group (Fig. 2A). In contrast MDA labeling was significantly low in the EtOH50 group (Fig. 2B).

3.4 Effects of DEC on TNF-α, IL-1β, COX-2 and iNOS expression in liver

Immunohistochemical analysis for TNF-α, a pro-inflammatory mediator in many experimental liver injury models, including alcohol-induced hepatitis, showed that this cytokine was highly induced by ethanol exposure (Fig. 2C), mainly in necrotic areas and macrophages. TNF-α immunopositivity decreased significantly in the livers of alcohol-induced mice treated with DEC (EtOH50) (Fig. 2D).

IL-1β is another cytokine that plays a role in inflammation and is required for the development of alcohol-induced liver injury. Immunohistochemical labeling for IL-1β in the alcoholic group (EtOH) showed high expression of this cytokine around the center-lobular veins, in the portal region and in macrophages (Fig. 2E). However, the group subjected to ethanol plus 50mg/kg DEC (EtOH50) had significantly reduced staining (Fig 2F). Confirming these results, Western blot (WB) for IL-1β showed that this interleukin was expressed in the control and DEC50 groups (Fig 3A). The chronic consumption of ethanol significantly increased protein expression and the group that received ethanol and was treated for 12 days with 50 mg/kg of DEC (EtOH50) had reduced IL-1β protein expression (Fig 3A).

COX-2 expression is related to the inflammatory phenomena present in chronic liver diseases. COX-2 was analyzed by immunohistochemical and WB. Immunostaining in hepatic tissue of the alcoholic group (EtOH) showed high expression of COX-2 in cytoplasm of hepatocytes (Fig.2G), which was significantly reduced in the hepatocytes of the EtOH50 group (Fig. 2H). WB showed that the high levels of COX-2 protein expression observed in the EtOH group (Fig 3B) were diminished after treatment with 50 mg/kg of DEC (EtOH50) (Fig 3B).

The inducible nitric oxide synthase (iNOS) gene is expressed by hepatocytes in a number of physiologic and pathophysiologic conditions. Immunostaining showed that the expression of iNOS was highly induced by ethanol exposure (Fig 2I) in cytoplasm of hepatocytes. The level of iNOS immunopositivity decreased significantly in the livers of alcoholic-induced mice treated with DEC (Fig 2J).

3.5 Effects of DEC on activation of NF-kB in liver

NF-κB is a nuclear transcription factor which, among other properties, regulates inflammatory genes. Immunohistochemical and WB analysis was performed with an

antibody against the p65 subunit of NF-κB and with phospho-NF-κB p65 antibody (pNF-κB) to evaluate the distribution of NF-κB in the hepatic tissue. A high level of NF-κB was seen in hepatocytes exposed to alcohol (Fig. 2K). pNF-κB positivity was found predominantly in the nucleus of hepatocytes. However immunopositivity in the cytoplasm of hepatocytes was also observed (Fig 2M). Levels of NF-κB and pNF-κB immunopositivity decreased significantly in the livers of alcoholic-induced mice treated with DEC (Fig 2L and 2N). In WB, no difference was observed in the expression of NF-κB protein in the cytoplasmatic fraction (Fig. 3D). In total and nuclear fractions, it was observed that the chronic consumption of ethanol significantly increased the protein expression of NF-κB and the group that received ethanol and was treated for 12 days with 50mg/kg of DEC (EtOH50) resulted in reduced expression of these proteins (Fig 3C and 3E). The same results were observed for pNF-κB of nuclear fraction (Fig 3F).

To determine whether the activation of NF-κB might be result of degradation of IκBα, protein expression of cytoplasmatic IκBα and pIκBα were measured using WB analysis. It was observed that the EtOH group had low expression of IκBα (Fig. 3G) and high expression of pIκBα (Fig. 3H), while the animals treated with DEC had high expression of IκBα (Fig 3G) and low expression of pIκBα (Fig. 3H).

4. Discussion

It is now widely accepted that the progression of liver injury consequent to chronic alcohol abuse is a multifactorial event that involves a number of genetic and environmental factors (Albano, 2006). The damage present in ALD involves cytokines, oxidases and protease genes that propagate responses through common signaling pathways involving NF-κB (Nanji et al., 1999). Defining mechanisms for liver

inflammation and cell death in ALD can provide new insights into the development of this process, and offer potential targets for therapeutic intervention.

Pharmacoterapy of liver disease can produce positive results, but can also have considerable negative effects. The clinical course of hepatic injury can be improved with the use of natural compounds with anti-oxidant properties (resveratrol), corticoids and anti-TNFα agents (pentoxifylline, infliximab, etanercept). However, more clinical data is necessary to standardize this treatment as these drugs can increase mortality, risk of infection and death of patients (Bruha et al, 2012; Gao and Bataller, 2011).

Few studies focus on the role of DEC in the pathophysiology of inflammation. According to Gonzalez et al. (1994) rats with hepatic inflammation induced by CCl_4 had an evident reduction of morphological damage after DEC treatment at 25 and 50 mg/kg, presenting well-preserved organelles and hepatic membrane system. In another study Rocha et al. (2012) demonstrated that DEC treatment at 50 mg/kg inhibited the hepatic injury and decreased inflammatory infiltration induced by ethanol consumption. In the present study, it was found that ingestion of ethanol (EtOH) for 5 weeks caused fatty liver, necrosis and inflammation and DEC treatment reduced hepatic injury, confirming previous studies.

The findings of the present study support the role of inflammatory mediators in alcohol liver damage. The EtOH group presented marked immunoexpression of MDA, IL-1β, TNF-α, COX-2, iNOS, NF-κB, pNF-κB. Treatment with DEC decreased the expression of these markers.

Several studies have suggested that ethanol may cause tissue damage through lipid peroxidation. The end products of the peroxidation of polyunsaturated fatty acids, such MDA, are used as markers to assess ROS-induced lipid peroxidation and may play roles in the pathogenesis of liver diseases (Sakaguchi et al, 2011). In the present study,

the increased MDA immunostaining observed in the EtOH group was reduced after DEC treatment, suggesting a possible antioxidant action.

Cytokines are mediators of cellular communication and in the liver they are produced by multiple cell types. Kupffer cells are prominent producers of proinflammatory cytokines, such as IL-1 and TNF-α (McClain et al., 2004). Chronic alcohol consumption, particularly in alcoholic liver disease, was shown to be associated with elevated levels of circulating TNF-α and IL-1 (Khoruts et al., 1991; McClain and Cohen, 1989). These cytokines promote infiltration of inflammatory leukocytes and activate oxidative responses, accompanied by a further release of cytokines and degradative proteins. (Hoec and Pastorino, 2002). The present study showed that DEC inhibited the release of IL-1β and TNF-α in alcoholic mice confirming previous studies that found that DEC decreased inflammatory markers in liver injury (Rocha et al., 2012).

COX-2 is considered to be a mitogen-inducible form, associated with biologic events such as injury, inflammation and proliferation (O' Banion et al., 1991, 1992; Kirschenbaum et al., 2000). COX-2 is induced by a variety of stimuli including IL-1, TNF-α, lipopolysaccharide (LPS), and oxidantive stress (Feng et al., 1994). One of the major eicosanoid products resulting in increased expression of COX-2 is thromboxanes (TXB2) (Crofford, 1997). Nanji et al (1994) showed the existent correlation between levels of thromboxane B2 (TXB2) in plasma of rats and the severity of ALD. Dinchuk et al (1995) observed that hepatocyte injury was markedly reduced in COX-2 knockout mice and that a combination of TNF-α and COX-2 was important in endotoxin-mediated hepatocellular injury.

Pharmacological studies have showed that DEC interferes with the arachidonic acid metabolism, acting as an anti-inflammatory drug. There is substantial information to suggest that DEC blocks a number of steps in both cyclooxigenase (COX) and lipoxigenase pathways, including inhibition of leucocyte chemotaxis, granulocyte degranulation, and peripheral vasodilation (Maizels and Denham 1992). Ours results demonstrated that DEC significantly diminishes the COX-2 activation present in the EtOH group confirming a possible anti-inflammatory action.

Nitric oxide (NO) is a potent vasorelaxant (Palmer et al., 1987), but also plays a role in physiological processes (Garthwaite et al., 1988) and host defense (Nathan and Hibbs, 1991). In the liver, NO is produced by constitutively expressed endothelial nitric oxide synthase (eNOS) or inducible nitric oxide synthase (iNOS). iNOS is negligible under physiological conditions, but is expressed in hepatic cells including hepatocytes and Kupffer cells under pathological conditions such as sepsis, hemorrhagic shock, ischemia-reperfusion, hepatitis, and cirrhosis. During infection and inflammation of the liver, excess production of NO by iNOS is thought to be involved in liver injury (Matsui et al., 2011).

Datta et al. (2006) suggest that iNOS/NO production has a regulatory effect on iNOS and COX-2 expression. The molecular regulation of iNOS expression is complex and occurs at multiple levels in the gene expression pathway. Several studies have demonstrated that the upregulation of iNOS is associated to the increase of COX-2 protein expression, and that NF-κB activation is essential for iNOS-dependent upregulation of COX-2 (Li et al 2003; 2007). Numerous mechanisms have evolved to regulate iNOS expression during hepatocellular injury. The cytokines TNF-α, IL-1β, and INF-γ synergistically activate iNOS expression in the liver, which is down-

regulated by steroids, TGF-β, the protein heat shock response, p53, and NO itself (Taylor et al., 1998). Tirapelli et al. (2011) observed in rats that chronic ethanol consumption increased the mRNA expression and immunostaining for iNOS, but not eNOS, indicating that ethanol up-regulates iNOS expression at the pre-translational level.

McGarry et al (2005) observed that iNOS and COX pathways were essentials for DEC activity *in vivo.* These authors showed that DEC was ineffective in *Brugia malayi* infected mice lacking iNOS and that DEC administration reduced the amount of COX-1 in the host. Queto et al (2010), in a study of pulmonary eosinophilic inflammation using OVA-sensitized BALB/c mice, showed that DEC suppressed allergen-induced eosinophilia in lungs and bone marrow through an iNOS/CD95L-dependent mechanism. In the present study, ethanol consumption increased iNOS and COX-2 expression and DEC treatment reduced these markers, confirming that the upregulation of iNOS is associated with increased COX-2 protein expression and that the inhibition of inflammation by DEC observed in the liver after chronic consumption of alcohol is iNOS-dependent.

NF-κB is one of the principal proinflammatory transcription factors (Tak et al 2001) and several studies have linked its action with liver damage caused by ethanol. Nanji et al. (1999) suggested that translocation of the transcripition factor NF-κB was associated with the development of necroinflammatory changes in the livers of alcohol-fed rats. In another study, the same authors observed that alcohol-induced liver disease was accompanied by the activation of NF-κB-dependent genes such as iNOS and COX-2. According to Zou and Crews, 2010 ethanol could initially activate NF-κB through reactive oxygen species and/or release of proinflammatory cytokines, such as TNF-α. In

the results of the present study it was observed that ethanol treated groups presented an increase of NF-κB and pNF-κB expression, cytokines related to its activation (IL-1β and TNF-α) and NF-κB-dependent markers (iNOS and COX-2).

Activation of NF-κB occurs secondarily to the proteolitic degradation of IκBα, allowing free NF-κB to translocate to the nucleus and initiate gene transcription (Baeuerle and Baltimore, 1996; May and Ghosh, 1997). The findings of the present study support a role for IκBα in alcoholic liver injury because the activation of NF-κB in the presence of inflammatory changes in the liver was accompanied by a loss of IκBα in the alcoholic treated group. The inhibition of NF-κB activation in the alcoholic group treated with DEC (EtOH50) was accompanied by the preservation of IκBα protein expression. The mechanisms involved in the stabilization of IκBα and suppression of NF-κB activation in the EtOH50 group by DEC remain to be elucidated, but a role for the NF-κB signaling cascade is suggested.

In the current study, it was found that treatment with DEC was highly effective in preventing experimental ALD. In addition to preventing alcohol-induced liver injury, it blocked lipid peroxidation, reduced the activation of NF-κB by stabilizing IκBα degradation and also reduced the expression of proinflammatory cytokines, iNOS and COX-2. These results suggest that DEC not only inhibits the hepatic local inflammatory response, but also attenuates the positive feedback loop between oxidative stress and inflammation. It is noteworthy that the anti-inflammatory properties of DEC are related not only to the inhibition of cyclooxygenases pathways, but also that it is involved in the inactivation of NF-κB signaling, which also synergistically activates COX-2 gene expression. The results here presented study indicate that DEC exerts potent anti-inflammatory effects by inhibiting both pathways. Also, it suggests that agents that

prevent the activation of a transcription factor, i.e., NF-κB, will suppress expression of a series of proinflammatory molecules and thereby prevent ALD.

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5. References

Achur, R. N., Freeman, W. M., Vrana, K. E., 2010. Circulating cytokines as biomarkers of alcohol abuse and alcoholism. J. Neuroimmune Pharmacol. 5, 83-91.

Albano, E., 2006. Alcohol, oxidative stress and free radical damage. Proc Nutr Soc 65, 278–290.

Arias-Salvatierra, D., Silbergeld, E.K., Acosta-Saavedra, L.C., Calderon-Aranda, E.S., 2011. Role of nitric oxide produced by iNOS through NF-kappaB pathway in migration of cerebellar granule neurons induced by Lipopolysaccharide. Cell Signal. 23, 425–435.

Baeuerle, P.A., Baltimore, D.,1996. NF-kappa B: Ten years after. Cell 87, 13–20.

Ballas, Z. K., Cook, R. T., Shey, M. R., Coleman, R. A., 2012. A Dynamic Flux in Natural Killer Cell Subsets as a Function of the Duration of Alcohol Ingestion. Alcohol. Clin. Exp Res. 36, 826-834.

Barnes, P. J., Karin, M. 1997. Nuclear factor-κB—a pivotal transcription factor in chronic inflammatory diseases. N. Engl. J. Med. 336: 1066-1071.

Bhaskaran, N., Shukla, S., Srivastava, J.K., Gupta, S., 2010. Chamomile: an anti-inflammatory agent inhibits inducible nitric oxide synthase expression by blocking RelA/p65 activity. Int. J. Mol. Med. 26, 935–940.

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.

Bruha, R., Dvorak, K., Petrtyl, J., 2012. Alcoholic liver disease. World J. Hepatol. 4, 81-90.

Cook, R.T., Zhua, X., Colemana, R.A., Ballas, Z.K., Waldschmidta, T.J., Raya, N.B.,Labrecqueb, D.R., Cook, B.L., 2004. T-cell activation after chronic ethanol ingestion in mice. Alcohol 33, 175–181.

Crofford, L. J. 1997. COX-1 and COX-2 tissue expression: implications and predictions. J. Rheumatol. *49*, 15-19.

Datta, P.K., Dhupar, S., Lianos, E.A., 2006. Regulatory effects of inducible nitric oxide synthase on cyclooxygenase-2 and heme oxygenase-1 expression in experimental glomerulonephritis. Nephrol. Dial Transplant. 21:51-7.

Dinchuk, J. E., Car, B. D., Focht, R. J., Johnston, J. J., Jaffee, B. D., Covington, M. B., et al. 1995. Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. Nature 378, 406 – 409.

Feng, L., Xia, Y., Garcia, G. E., Hwang, D., Wilson, C. B. 1995. Involvement of reactive oxygen intermediates in cyclooxygenase-2 expression induced by interleukin-1, tumor necrosis factor-alpha, and lipopolysaccharide. J. Clin. Invest. 95, 1669-1675.

Gao, B.; Bataller, R. 2011. Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology 141:1572-85.

Garthwaite, J., Charles, S. L., Chess-Williams, R. 1988. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. Nature 336**,** 385–388.

Gonzalez, R., Ancheta, O., M_arquez, M., Rodriguez, S., 1994. Hepatoprotective effects of diethylcarbamazine in acute liver damage induced by carbon tetrachloride in rats. Acta Pharmacol. Sin. 15: 495-497.

Hewitt, R.I., White, E., Wallace, W.S., Stewart, H.W., Kushner, S., Subbarow, Y., 1947. Experimental chemotherapy of filariasis; effect of piperazine derivatives against naturally acquired filarial infections in cotton rats and dogs. J. Lab. Clin. Med. 32, 1304-13.

Hoek, J. B., Pastorino, J. G., 2002. Ethanol, oxidative stress, and cytokine-induced liver cell injury. Alcohol, 27, 63-68.

Karinch, A. M., Martin, J. H., Vary, T. C., 2008. Acute and chronic ethanol consumption differentially impact pathways limiting hepatic protein synthesis. Am. J. Physiol. Gastrointest. Liver Physiol. 295 (1), E3-E9.

Khoruts, A., Stahnke, L., Mcclain, C.J., Logan, G, Allen, J. I., 1991. Circulating tumor necrosis factor interleukin-1 and interleukin-6 concentrations in chronic alcoholic patients. Hepatology, 13, 267–76.

Kirschenbaum, A., Liotta, D.R., Yao, S., Liu, X.H., Klausner, A.P., Unger, P., Shapiro, E., Leav, I., Levine, A.C., 2000. Immunohistochemical localization of cyclooxygenase-1 and cyclooxygenase-2 in the human fetal and adult male reproductive tracts. J. Clin. Endocr. Metab. 85, 3436–3441.

Li, Q., Guo, Y., Tan, W., Ou, Q., et al., 2007. Cardioprotection Afforded by Inducible Nitric Oxide Synthase Gene Therapy Is Mediated by Cyclooxygenase-2 via a Nuclear Factor-κB–Dependent Pathway. Circulation. 116, 1577-1584.

Li, Q., Guo, Y., Xuan, Y. T., Lowenstein, C. J., et al., 2003. Gene therapy with inducible nitric oxide synthase protects against myocardial infarction via a cyclooxygenase-2–dependent mechanism. Circ. Res. 92, 741-748.

Lieber, C. S., 2000. Alcohol and the liver: metabolism of alcohol and its role in hepatic and extrahepatic diseases. Mt. Sinai J. Med. 67, 84.

Lieber, C.S., 2005. Metabolism of Alcohol. Clin. Liver. Dis. 9, 1-35.

Maizels, R.M., Denham, D.A., 1992. Diethylcarbamazine (DEC): immunopharmacological interactions of an anti-filarial drug. Parasitology 105, Suppl:S49-60.

Mandrekar, P. Signaling mechanisms in alcoholic liver injury: Role of transcription factors, kinases and heat shock proteins. World J. Gastroenterol. 13: 4979-4985, 2007.

Matsui, K., Ozaki, T., Oishi, M., Tanaka, Y., et al., 2011. Active hexose correlated compound inhibits the expression of proinflammatory biomarker iNOS in hepatocytes. Eur. Surg. Res. 47, 274-283.

May, M.J., Ghosh, S., 1997. Rel/NF-κB and IκB proteins: an overview. Semin. Cancer Biol., Vol. 8, 63-73.

McClain, C. J., Song, Z., Barve, S. S., Hill, D. B., Deaciuc, I., 2004. Recent advances in alcoholic liver disease IV. Dysregulated cytokine metabolism in alcoholic liver disease. Am. J. Physiol Gastrointest. Liver Physiol, 287, G497-G502.

McClain, C.J., Cohen, D.A., 1989. Increased tumor necrosis factor production by monocytes in alcoholic hepatitis. Hepatology 9, 349–351.

McGarry, H. F., Plant, L. D., Taylor, M. J., 2005. Diethylcarbamazine activity against Brugia malayi microfilariae is dependent on inducible nitric-oxide synthase and the cyclooxygenase pathway. Filaria J. 4, 4.

Nanji, A.A., Jokelainen, K., Rahemtulla, A., Miao, L., Fogt, F., Matsumoto, H., Tahan, S.T., Su, G.L., 1999. Activation of nuclear factor kappa B and cytokine imbalance in experimental alcoholic liver disease in the rat. Hepatology, 30, 934–943.

Nanji, A. A., Khettry, U., Sadrzadeh, S. M., Yamanaka, T. 1993. Severity of liver injury in experimental alcoholic liver disease. Correlation with plasma endotoxin, prostaglandin E2, leukotriene B4, and thromboxane B2. The Am. J. Pathol. 142, 367-373.

Nathan, C.F., Hibbs, J.B.Jr., 1991. Role of nitric oxide synthesis in macrophage antimicrobial activity. Curr. Opin. Immunol. 3, 65–70.

O'Banion, M.K., Winn, V.D., Young, D.A., 1992. cDNA cloning and functional activity of a glucocorticoid-regulated inflammatory cyclooxygenase. Proc. Natl. Acad. Sci. 89, 4888–4892.

O'Banion, M.K., Sadowski, H.B., Winn, V., Young, D.A., 1991. A serumand glucocorticoid regulated 4-kilobase mRNA encodes a cyclooxygenase-related protein. J. Biol. Chem. 266, 23261–23267.

Palmer, R.M.J., Ferrige, A.G., Moncada, S., 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327, 524–526.

Queto, T., Xavier, P., Gardel, M.A., Luca, B., et al., 2010. Inducible nitric oxidesynthase/CD95L-dependent suppression of pulmonary and bone marrow eosinophilia by Diethylcarbamazine. Am. J. Respir. Crit. Care Med. 181, 429–437.

Rocha, S. W. S., Silva, B. S., Gomes, F. O. S., Silva, A. K. S., Raposo, C., Barbosa, K. P. S., Torres, D. O. C., Santos, AC. O., Peixoto, C. A., 2012. Effect of Diethylcarbamazine on chronic hepatic inflammation induced by alcohol in C57BL/6 mice. Eur. J. Pharmacol. 689, 194-203.

Sakaguchi, S., Takahashi, S., Sasaki, T., Kumagai, T., Nagata, K., 2011. Progression of alcoholic and non-alcoholic steatohepatitis: common metabolic aspects of innate immune system and oxidative stress. Drug Metab. Pharmacokinet. 26, 30-46.

Saraiva, K.L.A., Junior-Silva, V.A., Dias, E.S.F., Peixoto, C.A., 2006. Morphological changes in the testis induced by diethylcarbamazine. Reprod. Toxicol., 22, 754–759.

Tak, P.P., Firestein, G.S., 2001. NF-κB: a key role in inflammatory diseases. J. Clin. Inves. 107, 7-11.

Taylor, B. S., Alarcon, L. H., & Billiar, T. R. 1998. Reviews-Inducible Nitric Oxide Synthase in the Liver: Regulation and Function. Biochemistry-New York-English Translation of Biokhimiya 637, 766-781.

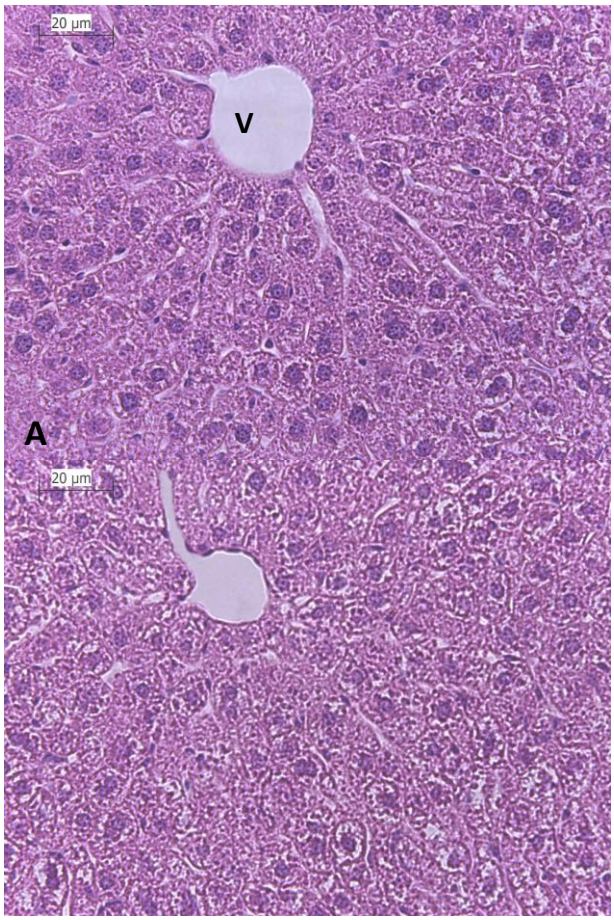
Terpstra, A.H.M., 2001. Differences between humans and mice in efficacy of the body fat lowering effect of conjugated linoleic acid: role of metabolic rate. J. Nutr. 131, 2067– 2068.

Tirapelli, L. F., Marcelo E. B., Ana L. JF., Daniela P. T., Evelin C. C., José E. TS., Regina H. Q., Sergio A. U., Cláudia M. P., Carlos R. T., 2011. Chronic ethanol consumption induces histopathological changes and increases nitric oxide generation in the rat liver. Tissue and Cell 43, 384-391.

World Health Organization. 2011. Global Status Report on Alcohol and Health. Geneva: WHO Press.

Zou, J., Crews, F., 2010. Induction of innate immune gene expression cascades in brain slice cultures by ethanol: key role of NF-kB and proinflammatory cytokines. Alcohol. Clin. Exp. Res. 34, 777–789.

FIGURE 1



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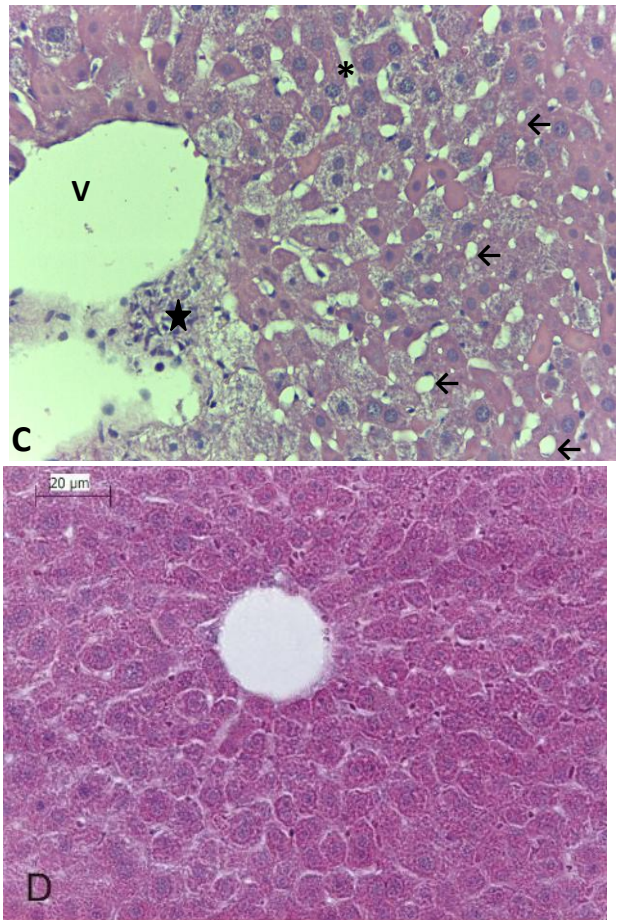
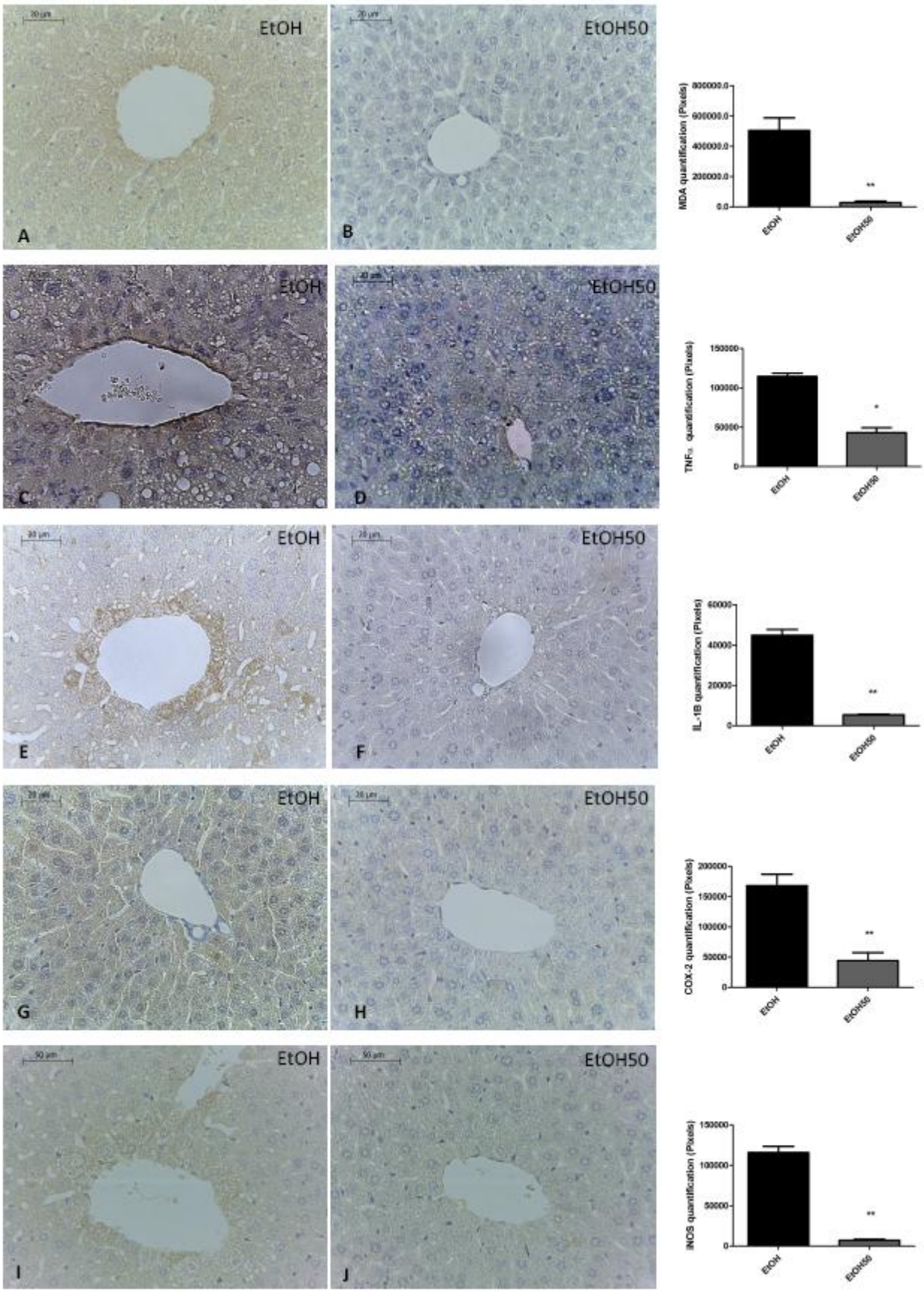


Figure 1. Micrograph of hepatocytes. **(A)** Liver parenchyma from control group showing typical morphology, **(B)** Group treated with 50mg/kg DEC (DEC50), **(C)** Alcoholic group (EtOH), **(D)** Alcoholic plus DEC group (EtOH50). Hepatic venule (V), lipid droplets (arrow), inflammatory infiltrates (star), cords of hepatocytes (asterisk). HE staining. Bars= 20 µm.

FIGURE 2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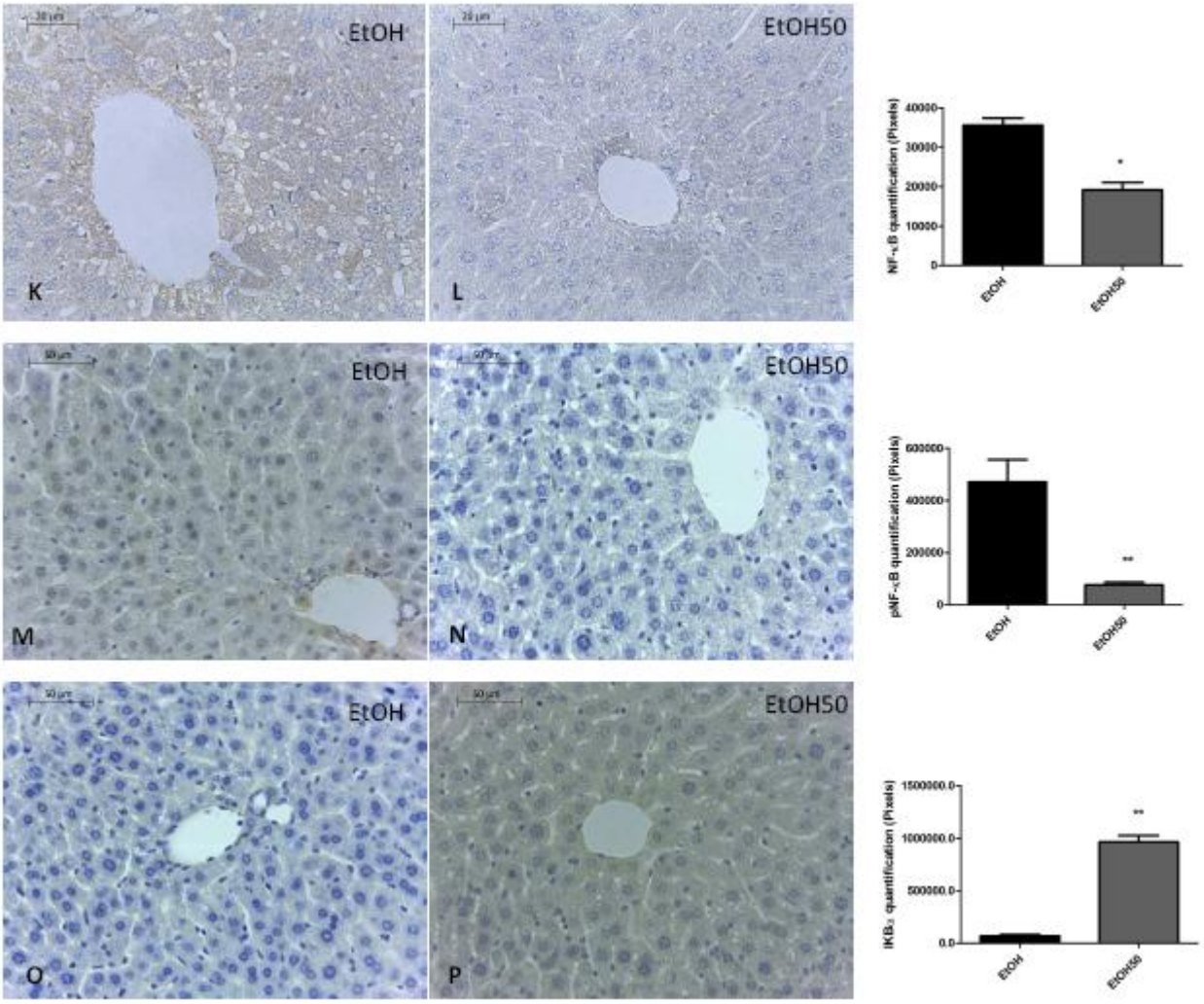

Figure 2. Immunohystochemical liver analysis. **(A)** MDA, **(C)** TNF-α, **(E)** IL-1β, **(G)** COX-2, **(I)** iNOS, **(K)** NF-κB, **(M)** pNF-κB and **(O)** IκBα in EtOH group. **(B)** MDA, **(D)** TNF-α, **(F)** IL-1β, **(H)** COX-2, **(J)** iNOS, **(L)** NF-κB, **(N)** pNF-κB and **(P)** IκBα in alcoholic plus DEC treated group. Intense labeling for MDA, TNF-α, IL-1β, COX-2, iNOS, NF-κB and pNF-κB present in the hepatocytes of the EtOH group. Reduced staining after DEC treatment for these markers was observed. EtOH group had low expression of IκBα, while the animals treated with DEC had high expression this enzyme. The quantification of cytokines (mean ± S.D., n= 5 in each group). **P* < 0.05, ***P* < 0.01 as compared with the control group. Bars= 20mm.

FIGURE 3

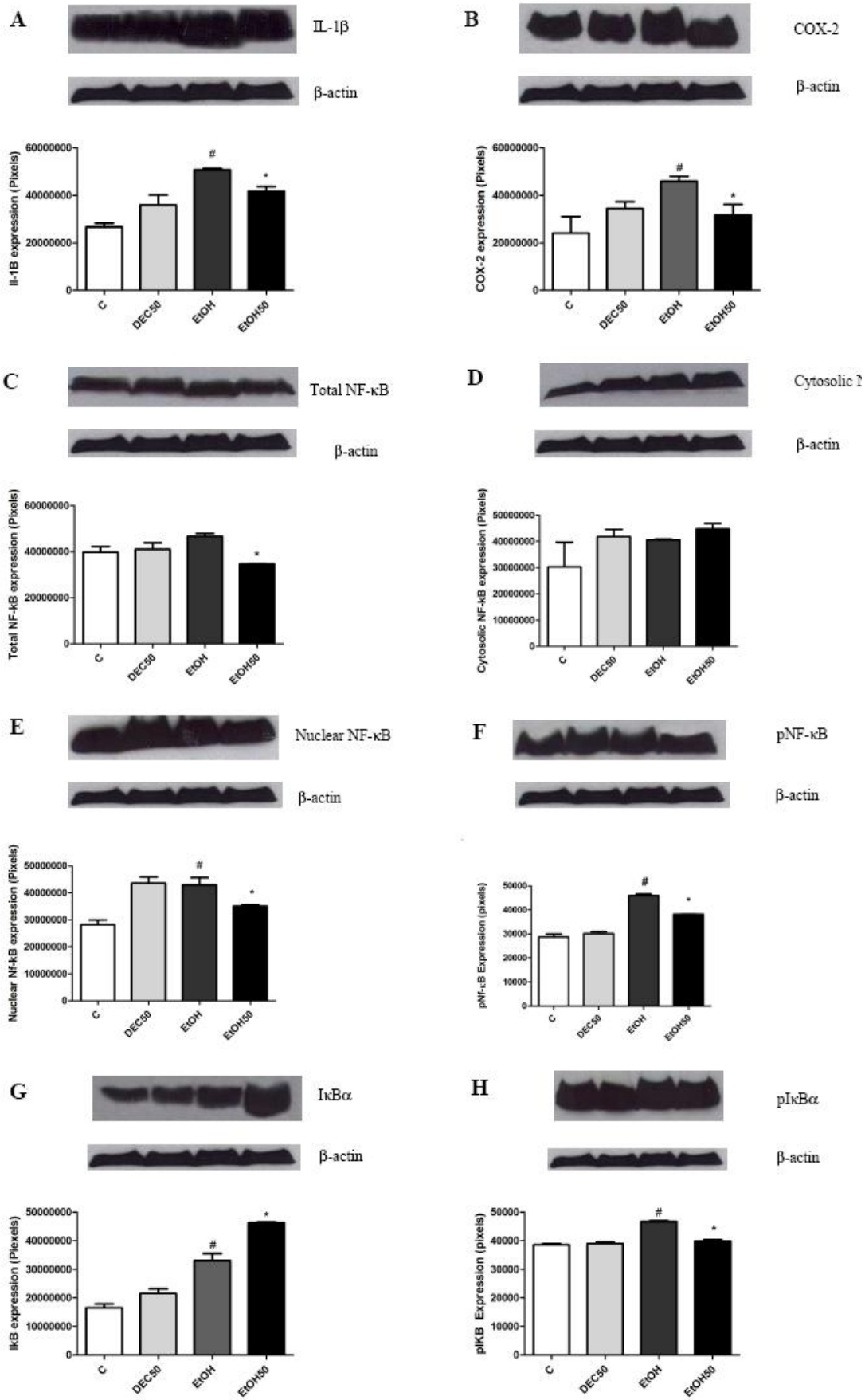
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Figure 3. Western blot analysis of protein liver expression. Protein extracts were used to analyze the expression of IL1-β **(A)**, COX-2 **(B)**, NF-κB total **(C)**, NF-κB cytosolic **(D)**, NF-κB nuclear **(E)**, pNF-κB nuclear **(F)**, IκBα **(G)** and pIκBα **(H)**. Chronic consumption of alcohol increased the expression of these markers. Alcoholic group treated with DEC inhibited NF-kB activity and expression of IL1-β, COX-2. No difference was observed in the protein expression of NF-κB cytosolic protein fraction **(D)**. Low expression Iκβα protein in EtOH group was observed while the alcoholic animals treated with DEC had a high expression of this enzyme **(H)**. $^{\#}$ $P < 0.05$, EtOH group when compared with control group. * $P < 0.05$, EtOH50 group when compared with EtOH group. Data was analyzed by *T* tests. Columns represent the mean ± S.D. of the protein investigated; results were confirmed in three sets of experiments (n= 4 animals for each group). β-actin was used as a control for the proteins blots.

CAPÍTULO III

ANTI-INFLAMMATORY EFFECTS OF DIETHYLCARBAMAZINE: A REVIEW

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Abstract

Diethylcarbamazine (DEC) is a well-known drug used throughout the world against lymphatic filariasis. However, along the last years, innumerous studies indicated other impressive pharmacological activities of DEC. Some preliminary clinical studies have stated that DEC was quite effective in asthmatic conditions, and various experimental studies used DEC as a potent leukotriene inhibitor. Nowadays, is well-established that DEC interferes with the cyclooxygenase pathways, reducing thromboxane, prostacyclin and prostaglandin production. Recent studies using different experimental models of inflammation indicated that DEC, besides inhibiting cyclooxygenases and lipoxygenases pathways, can also inhibit the nuclear transcription factor kappa B (NF-κB) activation, which is a key regulator of proinflammatory genes such us TNF-α, IL-1β, nitric oxide synthase inducible (iNOS) and even cyclooxygenase 2 (COX-2). This review will provide a comprehensive summary of DEC, including information on filaricidal action, inhibition of synthesis and secretory pathways, immunomodulatory activities, and specific inhibition of lipoxygenase and cyclooxygenase pathways.

1. Filaricidal Action

Diethylcarbamazine (DEC) has been used successfully as a principal public health tool to eliminate lymphatic filariasis in several countries throughout the world. Its filaricidal activity was discovered by Hewitt et al. [1] in wild cotton rats infected with *Litomosoiders sigmodontis.* Afterwards, Santiago-Steveson et al. [2] demonstrated its activity against *Wuchereria bancrofti* in human patients. Although DEC is the drug of choice for lymphatic filariasis, its mode of action is still a matter of controversy.

One of the most frequent findings is that DEC increases the microfilarial adherence to endothelial cells and granulocyte [3, 4, 5] which further biochemical

studies showed to be a result of inhibition of cyclooxygenases and lipoxygenases pathways [6, 7, 8]. These results led to the suggestion that DEC stimulates the innate arm of the immune system. In contrast, accumulated evidence indicates that microfilaricidal effect of DEC is not dependent on a specific humoral response. Weiner and Soulsby [9] showed that DEC reduced microfilariae levels by 95.8%, even when microfilariae of *Litomosoides sigmodontis* released in vitro were transfused into a naive animal, suggesting that an adaptive immune response was not a sufficient condition for DEC effectiveness. Similarly, Vickery et al. [10] showed that DEC mediates the clearance of *Brugia pahangi* microfilariae in immunodeficient nude mice.

Several other proposals suggested that DEC does not have a direct effect on the surface on the microfilariae, since some analyses revealed that the exposure of microfilariae to high concentrations of DEC left them unharmed; these results led to the idea that DEC had no direct effect on filarial parasites [5,11,12,13]. Barranco et al. [14] related paralysis of microfilariae treated *in vitro*, but unfortunately, the methodology consisted of imprecise DEC dilutions. However, in 2009, Rathaur et al. [15] incubated adult females worms and microfilariae of *Setaria cervi* with 100 µM of DEC, aspirin or indomethacin for 4 hours and observed that aspirin affected irreversibly the motility of both microfilariae and adult worms, while indomethacin and DEC were effective only on microfilarial stage with no significant effect on adult parasites even in higher concentrations. DEC treated microfilariae were straight, immobile and had wrinkled surface.

Ultrastructural studies developed in our laboratory showed drastic morphological damage to microfilariae of *Wuchereria bancrofti* after *in vitro* and *in vivo* treatment with DEC, indicating a possible direct mode of action. DEC promoted severe damage of microfilarial cells, including the presence of large vacuoles, lysis of

the cytoplasm and chromatin and bodies extruding from the plasma membrane, indicative features of an apoptotic process [16, 17, 18], which were confirmed by molecular tools as ligation-mediated polymerase chain reaction and in situ terminal deoxynucleotidyl transferase mediated dUTP nick end-labeling at light and electron transmission level [19]. However, it must be stressed that a convincing demonstration of an unequivocal pathway for direct mode of filarial killing by DEC has yet to be made, since the survival of microfilariae in serous cavities can be best explained by a mode of action in which helminthotoxic elements of the innate immune arm take part [20].

Pharmacological studies showed that DEC interferes with the arachidonic acid metabolism, acting as an anti-inflammatory drug. There are substantial information that DEC blocks a number of steps in both cyclooxygenase (COX) and lipoxygenase pathways, including inhibition of leucocyte chemotaxis, granulocyte degranulation, and peripheral vasodilation [21].

Filarial parasites also synthesize and release prostanoids, particularly prostacyclin and PGE2, which are vasodilators and potent platelet anti-aggregatory factors [22, 23, 24, 25, 26]. Further, it was also reported that DEC is a potent inhibitor of prostaglandin H synthase (cyclooxygenases), which is the first and rate-limiting enzyme in the transformation of polyunsatured fatty acids into prostaglandins, in filarial parasites [27]. Thereby, these data suggest that the mechanism by which DEC lowers the level of microfilariae in the circulation may in part involve its effects on host endothelial and parasite eicosanoid production.

In 2009, Rathaur et al. [15] showed that *Setaria cervi*, a bovine filarial parasite contains significant amounts of prostaglandin H synthase like enzyme (Sc-like-PGHS). They also provided experimental evidence that DEC, indomethacin and aspirin inhibit

Sc-like-PGHS at very lower concentrations, and hypothesized that microfilaricidal activity of DEC may partially depend on the inhibition of the parasitic PGHS.

Some studies demonstrated that nitric oxide (NO) plays an important role in host defense against filarial parasites *in vitro* [28, 29], but no evidence was found that DEC itself induces NO synthesis in murine macrophages and rat endothelial cells in vitro [30]. However, McGarry et al. [31] confirmed a lack of activity of DEC in mice deficient in iNOS infected with *B. malayi*, in addition to a reduced loss of COX-1 protein in peritoneal exudate cells. According to these authors, it seems that inducible NO is essential for rapid sequestration of microfilariae, and DEC probably stimulates its secretion via interaction with cyclooxygenase pathways. Recently, Sing and Rathaur [32] demonstrated that the exposure *in vitro* of the filarial parasite *Setaria cervi* to the combination of DEC plus aspirin at 100µM decreased PGHS activity leading to increase in NO level. By its turn, NO caused inhibition of tyrosine phosphatases, increasing the mitochondrial permeability through Bax, which allows cytocrome c to release in cytosol and activate caspases. Also, DNA fragmentation and ladder formation confirmed that adult worms *Setaria cervi* were killed by apoptosis. However, these effects were not observed when the worms were incubated with to aspirin or DEC alone.

In conclusion, taking into account the data obtained until now, is possible to suggest a direct mode of action of DEC on filarial worms mediated by the inhibition of cyclooxygenase pathways (PGHS) and consequent elevation of NO, which in high concentration, is a known inductor of mitochondrial-mediated apoptosis.

2. DEC acts as an inhibitor of synthesis and secretory pathways

DEC seems to inhibit synthesis and excretory activities in some cells. Ridge et al. [33] showed that macrophages in culture synthesize and secrete a soluble factor(s) that induces the synthesis of collagenase in primary cultures of rabbit chondrocytes.

Since macrophages are often present in inflammatory sites this would provide a possible mechanism of local connective tissue destruction. Other studies indicated that a lipooxygenase pathway of arachidonic metabolism is critical in activating various types of cells. Incubation of chondrocytes with MCM (Macrophage Conditioned Medium) and low doses of indomethacin (1-10 microM) had no effect on collagenase synthesis. However, the use of lipoxygenase inhibitors as NDGA (a nonspecific inhibitor) and DEC inhibited the synthesis of collagenase in chondrocytes. These inhibitors did not affect collagenase activity nor did they interfere with the activation of latent collagenase. These data indicated that although cyclooxygenase plays no role in the MCM dependent induction of collagenase in chondrocytes, lipoxygenase activity may be essential [34].

Similarly, in 1985, Stevens et al. [35] performed biochemical and morphological studies and demonstrated that DEC inhibited the synthesis and exocytosis of proteoglycan in rat chondrocytes. Treatment of chondrosarcoma chondrocytes with DEC altered the transport of molecules from the reticulum to the Golgi apparatus and the transport of molecules from the Golgi to the cell surface. After treatment with DEC, chondrocytes presented large vacuoles and a distended Golgi apparatus. Upon removal of DEC, the vacuoles disappeared and distended organelles returned to their normal appearance, coincident with the start of exocytosis of S-proteoglycan and β-D-xyloside-bound ^{35}S-glycosaminoglycan. Other studies confirmed that DEC disturbs the traffic of vesicles to and from the Golgi apparatus. Spiro et al. [36] demonstrated that DEC altered the vesicular transport from the endoplasmic reticulum to the Golgi, and from the Golgi to the plasma membrane inhibiting the surface expression of a melanoma associated chondroitin sulfate proteoglycan. In fetal mouse cerebellar neurons in culture DEC induced a complete disorganization of the Golgi apparatus and the appearance of

large vacuoles within the cytoplasm, suggesting that the various synthetic and secretory activities involving these organelles are impaired [37].

According to Fujimak et al. [38] studied the effect of DEC on microtubules by using microtubule protein prepared from porcine brain. DEC inhibited assembly of microtubules and disassembled preformed microtubules in vitro. DEC also inhibited the proliferation of LLC-MK2, and cells grown in the presence of DEC were likely to separate from each other and became round in shape. Immunofluorescence microscopy revealed that LLC-MK2 exposed to DEC were devoid of the delicate pattern of the cytoplasmic microtubule complex. Although these studies revealed an interesting activity of DEC as a possible inhibitor of some secretory pathways, we must be account that those were obtained with different kinds of cell lineages and drug concentrations. Further studies in vivo and/or ex vivo are necessary to confirm these observations.

3. DEC has an immunomodulatory activity

Different animal models and experimental approaches have been used for the assessment of DEC as a potential immunomodulator. Kitchen [39] related an immunomodulation effect of diethylcarbamazine on cats given feline leukaemia virus vaccine. Oral diethylcarbamazine when given with a retroviral vaccine, increased titres and duration of serum antibodies and 10-fold increased the peripheral monocyte counts in treated-vaccinated cats when compared to cats that received vaccine without diethylcarbamazine. Since macrophages are derived from monocytes and process novel antigens and thus are key cells for both humoral and cellular immune function, the described increased monocyte counts may signal increased immunity to vaccine antigens. Lately, Kitchen and Cotter [40] and Kitchen et al. [41] confirmed and extend these previous observations, demonstrating that treatment with DEC was associated

with decreased levels of circulating virus and prevented or delayed to develop lymphopenia in feline leukemia virus-infected cats.

Using another line of investigation, Medina De La Garza et al. [42] described an enhancing effect of DEC on eosinophils adherence to larvae of *Onchocerca volvulus* only in the presence of immune serum, but not in the presence of normal serum, indicating a possible involvement of DEC in modulation of effector cells. In a recent study, Medina De La Garza [43] demonstrated that DEC exerts a dose-dependent immunomodulatory effect. These authors evaluated the effect of DEC (50 and 500 mg/day) on antibody production, cytokine response and respiratory burst in BALB/c mice challenged with a thymus-dependent antigen (tetanus toxoid) and with a thymus-independent antigen (lipopolysaccharide). These authors concluded that DEC treatment enhanced antibody production in tetanus toxoid immunized mice, and cytokine response in LPS immunized animals. Also, the higher dose of DEC (500mg) induced respiratory burst of polymorphonuclear leucocytes and monocytes. According to these authors, DEC could act directly as potential immunomodulator or adjuvant for use in the treatment of human diseases.

4. DEC acts as specific lipoxygenase pathway inhibitor

Diethylcarbamazine is also of interest of other therapeutic role, as an anti-inflammatory drug for asthmatic conditions. In 1965, Salazar Mallén [44] reported very promising results in a preliminary trial with DEC in 14 out of 15 patients with intractable asthma without tropical eosinophilia. In 1970, Benner et al. [45] performed a double-blind study carried out in 18 patients with perennial asthma and did not find any significant differences between groups. In contrast, Srinivas and Antani [46] found that DEC was quite effective in the symptomatic treatment of 40 patients randomly selected

with an acute attack of bronchial asthma, reducing the amount of corticosteroids some of the patients had been taking and in some cases eliminating it altogether. Confirming this later study, Thiruvengadam et al. [47] conducted a double-blind in 50 patients with intractable bronchial asthma and concluded that DEC may be used in symptomatic relief and it could be a worthwhile addition to the armamentarium of drugs used in this disorder, if not entirely capable by itself of giving total symptom relief.

DEC like other drugs which inhibit antigen-induced mediator release in vitro such as sodium cromoglycate [48], and the beta-adrenoceptor agonists [49] prevents exercise induced-asthma in a significant proportion of asthmatic patients [50, 51, 52, 53]. González et al. [54] examined the pharmacological activity of this drug in several in vivo and in vitro models of experimental anaphylaxis. They stated that DEC at doses of 25 and 50 mg/kg given intraperitoneally significantly reduced the mortality rate in sensitized guinea pigs during protracted shock phase.

Lung ultrastructural studies demonstrated that after 12 days of treatment of mice with DEC induced significant alterations on type II pneumocytes and a substantially greater number of mature surfactant secretion vesicles, confirming that DEC exerts a role in the activation of important pulmonary cellular pathways, which are probably related to the clinical improvement of asthma symptoms after DEC treatment [55].

Leukotrienes (LTs), including cysteinyl LTs (CysLTs; these include LTC4, LTD4 and LTE4) and LTB4, are potent lipid mediators that have an important pathophysiological role in asthma and allergic rhinitis. LTs are potent lipid mediators derived from arachidonic acid through the 5-lipoxygenase (5-LO) pathway [56]. Initially, Orange et al. [57] demonstrated that DEC inhibits the release of slow-reacting substance of anaphylaxis (SRS-A) in the rat, before it was showed to be a mixture of cysteinyl-leukotrienes (CysLT). In 1982, Mathews and Murphy [6] demonstrated that

DEC inhibits the formation of LTB4 and cysteinyl-leukotrienes in mastocytoma cells while actually stimulating the formation of 5-hydroxyeicosatetraenoic acid (5-HETE), suggesting that the site of action of DEC in inhibiting leukotrienes formation may be the leukotrienes A_4 synthetase reaction. Similarly, results were obtained by Bach and Brashler [58] in rat basophil leukemia cells.

Under the experimental conditions DEC also attenuated the formation of LTC4 when macrophages were incubated with LPS, and prevented the cellular activation (enhanced phagocytosis) [59]. Histamine and serotonin are important mediators in the initiation and development of antigen-induced immediate asthmatic responses including air-way smooth muscle contraction and microvascular leakage. In 2001, Nomura et al. [60] demonstrated that in response to histamine and serotonin alveolar macrophages release chemotactic factors for inflammatory cells *in vitro*, which could be mainly attributed to LTB4. As other lipooxigenase inhibitors used in that study, DEC induced a significant decrease of the release of chemotatic factors from alveolar macrophages.

In an elegant study Stenmark et al. [61] tested the hypothesis that the monocrotaline would activate the acid arachidonic metabolism in rats, playing a role in the hypertensive monocrotaline injury. After 3 weeks of monocrotaline administration they found 6-ketoprostaglandin $F_{1\alpha}$, leukotrienes C_4, and thromboxane B_2 in lung lavages. Also, lung extracts contained CysCLs, including leukotriene D_4. DEC administration reduced the numbers and activity of inflammatory cells, blocked pulmonary hypertension, prevented right ventricular hypertrophy, and inhibited the CysCL production.

Similarly, Farber et al. [62] studying pulmonary hypertensive response to foreign body microemboli measured pulmonary hemodynamics and accumulation of

arachidonic acid metabolites in dogs during the infusion of indomethacin, as cyclooxygenase inhibitor, DEC as lipoxygenase inhibitor, and FPL 55712, a receptor antagonist for leukotriene C4/D4, and concluded that only DEC was effective in blocking the pulmonary hypertension.

Semb et al. [63] analyzing the role of leukotrienes in functional depression of isolated rat hearts demonstrated that DEC significantly inhibited the cardiodepressive effects of oxygen free radicals produced by activated polymorphonuclear granulocytes. In 2004, Zuo et al. [64] using DEC as specific 5-LOX blocker also demonstrated that DEC reduces significantly the release of superoxide anion radical in skeletal muscle.

Several other studies have utilized DEC as a specific leukotriene inhibitor, such as in acute allergic bronchoconstriction [65], hypoxia pulmonary vasoconstriction [66], acute intestinal inflammation [67], ethanol-injury to the gastric mucosa [68] , pathogenesis studies of bowel necrosis [69], and in experimental trauma-sepsis in rats by intravenous infusion of live *E. coli* [70].

More recently, Queto et al. [71] using OVA-sensitized BALB/c mice by airway demonstrated that DEC effectively prevented the effects of the subsequent challenges, such as airway resistance, Th1/Th2 cytokine production, pulmonary eosinophil accumulation and suppressed eosinophilopoiesis *in vivo* and *ex vivo.*

Is interesting to note that treatment with DEC of acute Tropical Pulmonary Eosinophilia (TPE), an interstitial lung disease that results from a heightened immunologic response to the human filarial parasites, *Wuchereria bancrofti* and *Brugia malayi*, leads to a marked rapid reduction of the eosinophilic alveolitis, decrease of peripheral blood eosinophilia and a concomitant increase in lung function. These observations are consistent with the concept that at least some of the abnormalities

found in the lung in acute TPE are mediated by an eosinophil-dominated inflammatory process in the lower respiratory tract [72, 73, 74].

5. DEC acts as cyclooxygenase pathway inhibitor

Although DEC is often designed as specific lipoxygenase inhibitor, several studies have demonstrated that this drug acts has also block some enzymatic steps of the cyclooxygenases pathways. Hagmann et al. [75] demonstrated that DEC blocked the lethal action of endotoxin in mice sensitized against lipopolysaccharide, and concluded that this effect was related to a lipoxygenase and cyclooxygenase inhibitor dual role of DEC. Other studies showed that DEC inhibited PGD2 and histamine release from purified human lung mast cells, indicating that this drug is responsible for pharmacologic modulation of arachidonic acid metabolites [76].

Piper and Stewart [77] studying the coronary vasoconstriction in the rat induced by platelet-activating factor, demonstrated that DEC (7.7mM) besides acting as lipoxygenase inhibitor, markedly attenuated the release of cyclooxygenase products as 6-keto-prostaglandin $F1_{\alpha}$ (6-keto-$PGF1_{\alpha}$), $PGF_{2\alpha}$, PGE_2 and thromboxane B_2 (TXB_2). Similarly, DEC exerts inhibition of the PGI2, 12-HETE, and 15-HETE synthesis in human endothelial cells [78]. In agreement with these previous results, El Tahir et al. [79] demonstrated that DEC has inherent property to inhibit the synthesis of the potent vasodilator, platelet antiaggregatory, anticonvulsant and antiinflammatory mediator PGI2 in thoracic aorta of rats, probably by interfering with the action of the PG endoperoxide synthase (PG cyclooxygenase).

The molecular cross-talk between iNOS and COX may regulate tissue homeostasis and contribute to pathophysiological processes. Some works had previously shown that there is a strict dependence of COX-2 up-regulation of iNOS [80,

81, 82, 83]. Further studies demonstrated that NF-kB activation is essential for iNOS-dependent upregulation of COX-2 [84].

McGarry et al. [31] observed that iNOS and COX pathways are essentials for DEC activity in vivo. These authors showed that DEC was ineffective in *Brugia malayi* infected mice lacking iNOS and also reduced the amount of the host's COX-1. Interestingly, Queto et al. [71] besides demonstrating that DEC has an efficient action as a leukotriene inhibitor in OVA-sensitized BALB/c mice model, reported that DEC had no effect on Th1/Th2 cytokine production, pulmonary eosinophil accumulation and eosinophilopoiesis suppress in CD95L-deficient gld mice, the ligand for apoptosis-inducing receptor CD95L (Fas), and in mice lacking iNOS activity. These results confirmed that DEC can possibly acts as an inductor of apoptosis as previously demonstrated by ultrastructural and molecular studies on microfilariae of W. Bancrofti [19].

Few studies focused the role of DEC in the pathophysiology of inflammatory conditions. According to Gonzalez et al. [85], rats with hepatic inflammation induced by CCl4 had an evident reduction of morphological damage after DEC treatment at 25 and 50 mg/kg, presenting well-preserved organelles and hepatic membrane system. Using the same doses, Rocha et al. [86] prevented the hepatic lipid accumulation and the damage caused by malnutrition after treatment of malnourished mice with DEC.

Recently a study performed in our laboratory analyzed the anti-inflammatory effect of DEC on hepatic cells of alcoholic mice. The DEC-treated group, which received 50 mg/kg for 12 day (a dose equivalent to 6mg/kg given to human preconized by OMS) reduced significantly several parameters of chronic hepatic inflammation, as steatosis, necrosis and foci of inflammatory infiltrates, serum AST levels and inflammatory markers as malondialdehyde (MDA), NF-kB, tumor necrosis factor-α

(TNF-α), interleukin-6 (IL-6), vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule (ICAM-1), monocyte chemotactic peptide-1 (MCP-1) and its functional receptor C2 chemokine receptor (CCR2), and inducible nitric oxide synthase (iNOS) in hepatic tissue [87].

Similar results were obtained in our laboratory using another experimental model. We evaluated the anti-inflammatory activity of DEC in a mouse model of acute lung inflammation (carrageenan-induced pleurisy). The injection of carrageenan into the pleural cavity induced the accumulation of fluid containing a large number of polymorphonuclear cells (PMNs) as well as infiltration of PMNs in lung tissues and increased production of nitrite, tumor necrosis factor-α (TNF-α), increased expression of interleukin-1β (IL-1β), cyclooxygenase (COX-2), inducible nitric oxide synthase (iNOS) and nuclear transcription factor kappa B (NFκB). The oral administration of DEC (50 mg/Kg) three days prior to the carrageenan challenge led to a significant reduction in all inflammation markers (unpublished works). There is ample evidence in carrageenan and other models of inflammation that the enhanced formation of prostanoids following the induction of COX-2 contributes to the pathophysiology of local inflammation [88, 89] and that selective inhibitors of COX-2 exert potent anti-inflammatory effects.

Is important to note that besides inhibiting lipoxygenases and cyclooxygenases DEC can also blocks the NFκB activation (Figure 1). Experimental evidence have clearly suggested that NFκB plays a central role in the regulation of many genes responsible for the generation of mediators or proteins in acute lung inflammation associated with carrageenan administration such us TNF-α, IL-1β, nitric oxide synthase inducible (iNOS) and COX-2. Therefore, the inhibition of the liberation of TNF-α and IL-1β by DEC could be attributed to the inhibitory effects of the activation of NFκB.

Further studies are in development towards better understanding of the biochemical mechanisms that underlie the poorly understood actions of this drug.

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7. References

1. Hewitt RI, White E, Wallace, WS, Stewart HW, Kushner S, Subbarow Y. Experimental chemotherapy of filariasis; effect of piperazine derivatives against naturally acquired filarial infections in cotton rats and dogs. J Lab Clin Med. 1947;32:1304-13.

2. Santiago-Stevenson D, Oliver-Gonzales J, Hewitt RI. The treatment of filariasis bancrofti with 1-diethylcarbamyl-4-methylpiperazine hydrochloride, hetrazan. Ann N Y Acad Sci. 1948;25:161-70.

3. Piessens WF, Beldekas M. Diethylcarbamazine enhances antibody-mediated cellular adherence to Brugia malayi microfilariae. Nature. 1979;282:845-7.

4. Rácz P, Tenner-Rácz K, Büttner DW, Albiez EJ. Ultrastructural evidence for eosinophil-parasite adherence (EPA) reaction in human onchocercal

lymphadenitis in the early period following diethylcarbamazine treatment. Tropenmed Parasitol. 1982;33:213-8.

5. Johnson P, Mackenzie CD, Denham DA, Suswillo RR. The effect of diethylcarbamazine on the in vitro serum-mediated adherence of feline granulocytes to microfilariae of Brugia pahangi. Trop Med Parasitol. 1988;39:291-4.

6. Mathews WR, Murphy RC. Inhibition of leukotriene biosynthesis in mastocytoma cells by diethylcarbamazine. Biochem Pharmacol. 198;31:2129-32.

7. Razin E, Romeo LC, Krilis S, Liu FT, Lewis RA, Corey EJ, Austen KF. An analysis of the relationship between 5-lipoxygenase product generation and the secretion of preformed mediators from mouse bone marrow-derived mast cells. J Immunol. 1984;133:938-45.

8. Ogletree ML. Overview of physiological and pathophysiological effects of thromboxane A_2. Fed Proc. 1987;46:133-8.

9. Weiner DJ, Soulsby EJ. Litomosoides carinii: effect of diethylcarbamazine on microfilaremias of Mastomys natalensis harboring old infections, new infections, and transfused microfilariae. J Parasitol. 1982;68:1105-9.

10. Vickery AC, Nayar JK, Tamplin ML. Diethylcarbamazine-mediated clearance of Brugia pahangi microfilariae in immunodeficient nude mice. Am J Trop Med Hyg. 1985;34:476-83.

11. Hawking F, Laurie W. Action of hetrazan on filariasis an onchocerciasis. Lancet. 1949;2:146.

12. Hawking F, Sewell P, Thurston JP. The mode of action of hetrazan on filarial worms. Br J Pharmacol Chemother. 1950 Jun;5(2):217-38.

13. Hawking F. Diethylcarbamazine and new compounds for the treatment of filariasis. Adv Pharmacol Chemother. 1979;16:129-94.

14. Barranco DGQFB, Fernández TAQBP, Zamora AC, Mállen MS. Effectos “in vitro” de la dietilcarbamazina sobre Onchocerca volvulus. Salud Publica 1962;6:1079-1082.

15. Rathaur S, Singh A, Yadav M, Rai R. Evidence for the presence of prostaglandin H synthase like enzyme in female Setaria cervi and its inhibition by diethylcarbamazine. Acta Trop. 2009;111:71-7.

16. Florêncio MS, Peixoto, CA. The effects of diethylcarbamazine on the ultrastructure of microfilarie of *Wuchereria bancrofti*. Parasitol. 2003;26:551-554.

17.Peixoto CA, Alves LC, Brayner FA, Florêncio MS. Diethylcarbamazine induces loss of microfilarial sheath of Wuchereria bancrofti. Micron. 2003;34:381-5.

18. Peixoto CA, Rocha A, Aguiar-Santos A, Florêncio MS. The effects of diethylcarbamazine on the ultrastructure of microfilariae of Wuchereria bancrofti in vivo and in vitro. Parasitol Res. 2004;92:513-7.

19. Peixoto CA, Santos AC, Ayres CF. Molecular evidence for apoptosis in microfilariae of Wuchereria bancrofti induced by diethylcarbamazine. Parasitol Res. 2008;103:717-21.

20. Cesbron JY, Capron A, Vargaftig BB, Lagarde M, Pincemail J, Braquet P, Taelman H, Joseph M. Platelets mediate the action of diethylcarbamazine on microfilariae. Nature. 1987;325:533-6.

21.Maizels RM, Denham DA. Diethylcarbamazine (DEC): immunopharmacological interactions of an anti-filarial drug. Parasitology. 1992;105 Suppl:S49-60.

22. Liu LX, Serhan CN, Weller PF.Intravascular filarial parasites elaborate cyclooxygenase-derived eicosanoids. J Exp Med. 1990;172:993-6.

23. Liu LX, Weller PF. Arachidonic acid metabolism in filarial parasites. Exp Parasitol 1990; 71:496-501.

24. Liu LX, Weller PF. Intravascular filarial parasites inhibit platelet aggregation. Role of parasite-derived prostanoids. J Clin Invest. 1992;89:1113-20.

25. Sommer A, Rickert R, Fischer P, Steinhart H, Walter RD, Liebau E. A dominant role for extracellular glutathione S-transferase from Onchocerca volvulus is the production of prostaglandin D2. Infect Immun. 2003;71:3603-6.

26. Brattig NW, Schwohl A, Rickert R, Büttner DW. The filarial parasite Onchocerca volvulus generates the lipid mediator prostaglandin E(2). Microbes Infect. 2006;8:873-9.

27. Kanesa-thasan N, Douglas JG, Kazura JW. Diethylcarbamazine inhibits endothelial and microfilarial prostanoid metabolism in vitro. Mol Biochem Parasitol. 1991;49:11-9.

28. Rajan TV, Porte P, Yates JA, Keefer L, Shultz LD. Role of nitric oxide in host defense against an extracellular, metazoan parasite, Brugia malayi. Infect Immun. 1996;64:3351-3.

29. Taylor MJ, Cross HF, Mohammed AA, Trees AJ, Bianco AE. Susceptibility of Brugia malayi and Onchocerca lienalis microfilariae to nitric oxide and hydrogen peroxide in cell-free culture and from IFN gamma-activated macrophages. Parasitology. 1996;112:315-22.

30. Rajan TV, Shultz LD, Babu S, Doukas J, Greiner D, Porte P. Diethylcarbamazine (DEC) does not induce nitric oxide (NO) synthesis. Exp Parasitol. 1998;88:217-22.

31. McGarry HF, Plant LD, Taylor MJ. Diethylcarbamazine activity against Brugia malayi microfilariae is dependent on inducible nitric-oxide synthase and the cyclooxygenase pathway. Filaria J. 2005;4:4.

32. Singh A, Rathaur S. Combination of DEC plus aspirin induced mitochondrial mediated apoptosis in filarial parasite *Setaria cervi*. Biochimie. 2010;92:894-900.

33. Ridge SC, Oronsky AL, Kerwar SS.Induction of the synthesis of latent collagenase and latent neutral protease in chondrocytes by a factor synthesized by activated macrophages. Arthritis Rheum. 1980;23:448-54.

34. Nolan JC, Pickett WC. Studies on the effects of cyclo-oxygenase and lipoxygenase inhibitors on the macrophage stimulated synthesis of collagenase by rabbit chondrocytes. Agents Actions. 1985;17:73-6.

35. Stevens RL, Parsons WG, Austen KF, Hein A, Caulfield JP. Novel inhibition of proteoglycan synthesis and exocytosis by diethylcarbamazine in the Swarm rat chondrocyte. J Biol Chem. 1985;260:5777-86.

36. Spiro RC, Parsons WG, Perry SK, Caulfield JP, Hein A, Reisfeld RA, Harper JR, Austen KF, Stevens RL. Inhibition of post-translational modification and surface expression of a melanoma-associated chondroitin sulfate proteoglycan by diethylcarbamazine or ammonium chloride. J Biol Chem. 1986;261:5121-9.

37. Bird MM. The effects of diethylcarbamazine on the morphology and ultrastructure of foetal mouse cerebellar neurons in culture. J Electron Microsc 2000;49:669–74.

38. Fujimarki Y, Ehara M, Kimura E, Shimida M, Aoki Y. Diethylcarbamazine, antifilarial drug, inhibits microtubule polymerization and disrupts preformed microtubules. Biochem Pharmacol 1990;39:851–6.

39. Kitchen LW. Effect of diethylcarbamazine on cats given feline leukaemia virus vaccine. Vaccine 1987;5:266-267.

40. Kitchen LW, Cotter SM. Effect of diethylcarbamazine on serum antibodyto feline oncornavirus-associated cell membrane antigen in feline leukemia virus cats. J Clin Lab Immunol 1988;25:101-103.

41. Kitchen LW, Mather F J, Cotter SM. Effect of continuous oral diethylcarbamazine treatment on lymphocyte counts of feline leukemia virus-infected cats. J Clin Lab Immunol 1988;27:179-181.

42. Medina-De la Garza CE, Brattig NW, Tischendorf FW, Jarrett JM. Serum-dependent interaction of granulocytes with Onchocerca volvulus microfilariae in generalized and chronic hyper-reactive onchocerciasis and its modulation by diethylcarbamazine. Trans R Soc Trop Med Hyg. 1990;84:701-6.

43. Medina-De la Garza CE, Guerrero-Ramírez G, García-Hernández M, Castro-Corona MA, Torres-López E, Brattig NW, Salinas-Carmona MC. Immunomodulatory activity of diethylcarbamazine on humoral, cellular cytokine response and respiratory burst in BALB/c mice. Immunopharmacol Immunotoxicol. 2012;34:477-83.

44. Salazar Mallén M. Treatment of intractable asthma with diethylcarbamazine citrate. Ann Allergy. 1965;23:534-7.

45. Benner M, Lowell FC. Failure of diethylcarbamazine citrate (Hetrazan) in the treatment of asthma. J Allergy. 1970;46:29-31.

46. Srinivas HV, Antani J. Diethylcarbamazine in bronchial asthma. Ann Allergy. 1971;29:418-21.

47. Thiruvengadam KV, Subramaniam N, Devarajan TV, Zachariah MG. Diethylcarbamazine citrate in bronchial asthma. J Indian Med Assoc. 1974;63:278-81.

48. Cox JSG. Disodium cromoglycate (FPL 670) (Intal) a specific inhibitor of reaginic antibody antigenmechanism. Nature 1967; 216:1328-1329.

49. Assem ES, Schild HO. Inhibition by sympathomimetic amines of histamine release by antigen in passively sensitized human lung. Nature 1969;224:1028-9.

50. Sly RM, Matzen K. Effect of diethylcarbamazine pamoate upon exercise-induced obstruction in asthmatic children. Ann Allergy 1974; 33:138-44.

51. Anderson SD, Seale JP, Ferris L, Schoeffel R, Lindsay DA. Evaluation of pharmacotherapy in exercise-induced asthma. J Allergy Clin Immunol 1979;64:612-624.

52. Anderson S D , Bye P T P, SchoeffeL R E, Seale J P, Taylor K M and Ferris L. Arterial plasma histamine levels at rest, and during and after exercise in patients with asthma: effects of terbutaline aerosol. Thorax.1981;36:259-267.

53. Li AR. An experimental study on the treatment of asthmatics with diethylcarbamazine. Zhonghua Jie He He Hu Xi Za Zhi 1991;14:208-10, 254.

54. González R, García M, De La Vega AR, Arruzazabala ML, Pérez H. Actions of diethylcarbamazine in experimental anaphylaxis. Allergol Immunopathol. 1979;7:125-34.

55. Florêncio MS, Saraiva KL, Peixoto CA. The effects of diethylcarbamazine on the ultrastructure of lung cells in vivo. Tissue Cell. 2005;37:241-6.

56. Montuschi P, Sala A, Dahlén SE, Folco G. Pharmacological modulation of the leukotriene pathway in allergic airway disease. Drug Discov Today. 2007;12:404-12.

57. Orange RP, Valentine MD, Austen KF. Inhibition of the release of slow-reacting substance of anaphylaxis in the rat with diethylcarbamazine. Proc Soc Exp Biol Med. 1968;127:127-32.

58. Bach MK, Brashler JR. Inhibition of the leukotriene synthetase of rat basophil leukemia cells by diethylcarbamazine, and synergism between diethylcarbamazine and piriprost, a 5-lipoxygenase inhibitor. Biochem Pharmacol. 1986;35:425-33.

59. Schade UF. Involvement of lipoxygenases in the activation of mouse macrophages by endotoxin. Biochem Biophys Res Commun. 1986;138:842-9.

60. Nomura H, Sato E, Koyama S, Haniuda M, Kubo K, Nagai S, Izumi T. Histamine stimulates alveolar macrophages to release neutrophil and monocyte chemotactic activity. J Lab Clin Med. 2001;138:226-35.

61. Stenmark KR, Morganroth ML, Remigio LK, Voelkel NF, Murphy RC, Henson PM, Mathias MM, Reeves JT. Alveolar inflammation and arachidonate metabolism in monocrotaline-induced pulmonary hypertension. Am J Physiol. 1985;248:H859-66.

62. Farber HW, Fairman RP, Rounds S, Millan JE, Glauser FL. Pulmonary hypertensive response to foreign body microemboli. Prostaglandins Leukot Essent Fatty Acids. 1990;39:151-7.

63. Semb AG, Vaage J, Mjøs OD. Oxygen free radical producing leukocytes cause functional depression of isolated rat hearts: role of leukotrienes. J Mol Cell Cardiol. 1990 May;22(5):555-63.

64. Zuo L, Christofi FL, Wright VP, Bao S, Clanton TL. Lipoxygenase-dependent superoxide release in skeletal muscle. J Appl Physiol. 2004;97:661-8.

65. Vargas MH, Montaño LM, Vanda B, Segura P, Selman M. Pharmacological characterization of mediators and vagal influence in the acute allergic

bronchoconstriction in guinea pigs. Naunyn Schmiedebergs Arch Pharmacol. 1990;342:278-83.

66. Mayers I, Johnson D, Wobeser W, To T. Relative roles of prostaglandins and leukotrienes in canine hypoxic pulmonary vasoconstriction. Clin Invest Med. 1991;14:44-54.

67. Woolverton CJ, White JJ Jr, Sartor RB.Eicosanoid regulation of acute intestinal vascular permeability induced by intravenous peptidoglycan-polysaccharide polymers. Agents Actions. 1989;26:301-9.

68. Balaa MA, Subramony C. Diethylcarbamazine decreases ethanol-injury to the gastric mucosa of the rat. Eicosanoids. 1990;3:201-3.

69. Hsueh W, Gonzalez-Crussi F, Arroyave JL. Platelet-activating factor-induced ischemic bowel necrosis. An investigation of secondary mediators in its pathogenesis. Am J Pathol. 1986;122:231-9.

70. Hall-Angerås M, Säljö A, Hasselgren PO. Effect of methylprednisolone, indomethacin, and diethylcarbamazine on survival rate following trauma and sepsis in rats. Circ Shock. 1986;20:231-8.

71. Queto T, Xavier P, Gardel MA, Luca, B. et al. Inducible nitric oxidesynthase/CD95L-dependent suppression of pulmonary and bone marrow eosinophilia by Diethylcarbamazine. Am. J. Respir. Crit. Care Med. 2010; 181:429–437.

72. Pinkston P, Vijayan VK, Nutman TB, Rom WN, O'Donnell KM, Cornelius MJ, Kumaraswami V, Ferrans VJ, Takemura T, Yenokida G. Acute tropical

pulmonary eosinophilia. Characterization of the lower respiratory tract inflammation and its response to therapy. J Clin Invest. 1987;80:216–225.

73. Rom WN, Vijayan VK, Cornelius MJ, Kumaraswami V, Prabhakar R, Ottesen EA, Crystal RG. Persistent lower respiratory tract inflammation associated with interstitial lung disease in patients with tropical pulmonary eosinophilia following conventional treatment with diethylcarbamazine. Am Rev Respir Dis. 1990;142:1088-92.

74. Vijayan VK. Tropical pulmonary eosinophilia: pathogenesis, diagnosis and management.Curr Opin Pulm Med. 2007;13:428-33.

75. Hagmann W, Denzlinger C, Keppler D. Role of peptide leukotrienes and their hepatobiliary elimination in endotoxin action. Circ Shock. 1984;14:223-35.

76. Peters SP, MacGlashan DW Jr, Schleimer RP, Hayes EC, Adkinson NF Jr, Lichtenstein LM. The pharmacologic modulation of the release of arachidonic acid metabolites from purified human lung mast cells. Am Rev Respir Dis. 1985;132:367-73.

77. Piper PJ, Stewart AG. Coronary vasoconstriction in the rat, isolated perfused heart induced by platelet-activating factor is mediated by leukotriene C4. Br. J. Pharmac. 1986;88: 595-605.

78. Ibe BO, Falck JR, Johnson AR, Campbell WB. Regulation of synthesis of prostacyclin and HETEs in human endothelial cells. Am J Physiol. 1989;256:C1168-75.

79. El Tahir KE, Al-Kharji AM, Ageel AM. Influence of diethylcarbamazine and mefloquine on PGI2 synthesis by the rat thoracic aorta and myometrial tissues. Gen Pharmacol. 1991;22:837-46.

80. Shinmura K, Xuan YT, Tang XL, Kodani E, Han H, Zhu Y, Bolli R. Inducible nitric oxide synthase modulates cyclooxygenase-2 activity in the heart of conscious rabbits during the late phase of ischemic preconditioning. Circ Res. 2002;90:602-8.

81. Li Q, Guo Y, Xuan YT, Lowenstein CJ, Stevenson SC, Prabhu SD, Wu WJ, Zhu Y, Bolli R.Gene therapy with inducible nitric oxide synthase protects against myocardial infarction via a cyclooxygenase-2-dependent mechanism. Circ Res. 2003;92:741-8.

82. Virdis A, Colucci R, Fornai M, Blandizzi C, Duranti E, Pinto S, Bernardini N, Segnani C, Antonioli L, Taddei S, Salvetti A, Del Tacca M. Cyclooxygenase-2 inhibition improves vascular endothelial dysfunction in a rat model of endotoxic shock: role of inducible nitric-oxide synthase and oxidative stress. J Pharmacol Exp Ther. 2005;312:945-53.

83. Jiang X, Shi E, Nakajima Y, Sato S. COX-2 mediates morphine-induced delayed cardioprotection via an iNOS-dependent mechanism. Life Sci. 2006;78:2543-9.

84. Li Q, Guo Y, Tan W, Ou Q, Wu WJ, Sturza D, Dawn B, Hunt G, Cui C, Bolli R. Cardioprotection afforded by inducible nitric oxide synthase gene therapy is mediated by cyclooxygenase-2 via a nuclear factor-kappaB dependent pathway. Circulation. 2007;116:1577-84.

85. Gonzalez R, Ancheta O, Marquez M, Rodriguez S. Hepatoprotective effects of diethylcarbamazine in acute liver damage induced by carbon tetrachloride in rats. Zhongguo Yao Li Xue Bao 1994;15: 495–497.

86. Rocha SWS, Santos ACO, Silva BS, Torres DOC, Ribeiro EL, Barbosa KPS, Gomes FO, Peixoto CA. Effects of diethylcarbamazine (DEC) on hepatocytes of C57BL/6J mice submitted to protein malnutrition. J. Food Drug Anal. 2012;20 (2).

87. Rocha SWS, Silva BS, Gomes FO, Soares e Silva AK, Raposo C, Barbosa KP, Torres D. de O, dos Santos AC, Peixoto CA. Effect of diethylcarbamazine on chronic hepatic inflammation induced by alcohol in C57BL/6 mice. Eur J Pharmacol. 2012;689:194-203.

88. Salvemini D, Manning PZ, Zweifel BS, Seibert K, Connor J, Currie MG, Needleman P, Masferrer JL. Dual inhibition of nitric oxide and prostaglandin production contributes to the antiinflammatory properties of nitric oxide synthase inhibitors. J Clin Invest 1995; 96:301-308.

89. Sautebin L, Ialenti A, Di Rosa M. Relationship between nitric oxide and prostaglandins in carrageenan pleurisy. Biochem Pharmacol 1998; 55:1113-7.

FIGURE 1

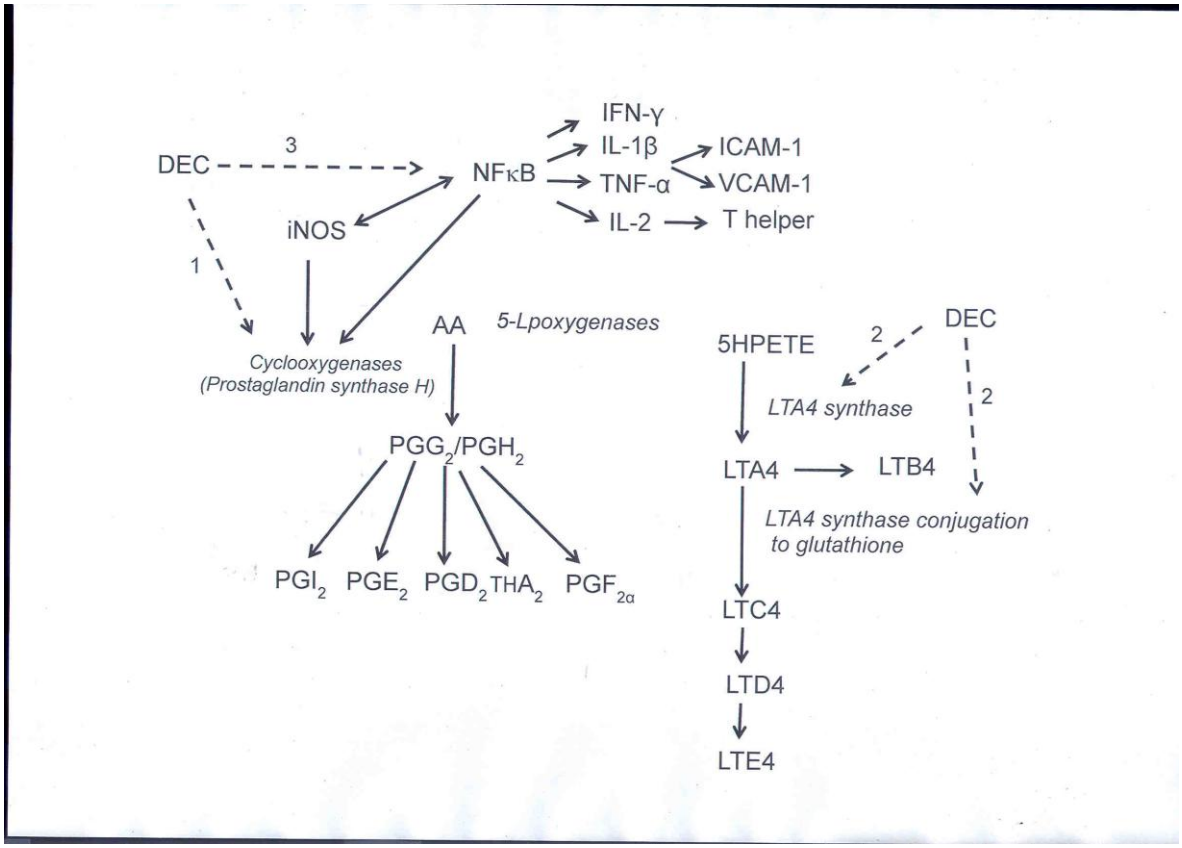


Fig 1. Schematic diagram showing the hypothetic involvement of DEC on inflammatory pathways. DEC can inhibit directly cyclooxigenases (COX-1, COX-2) enzymatic activity or can indirectly down-regulate COX expression by inhibiting the nuclear transcription factor NFkB. Inhibition of lipoxigenase pathway blockage LTB4 and cystenil-leukotrienes synthesis. Enzymes are named in italics. Dashed lines indicate inhibitory activity of DEC. Numbers indicate related references: (1) Kanesa-thasan et al., 1991; (2) Razin et al., 1984; Mathews and Murphy, 1982; (3) Rocha et al., 2010

APÊNDICES

APÊNDICE 1: RESULTADOS ADICIONAIS – ARTIGO 3

INTRODUÇÃO

O metabolismo do etanol ocorre primariamente no fígado e a consequência do etanol na função hepática pode ser dependente e/ou independente do metabolismo do etanol pelos hepatócitos (NAGY, 2004). A injúria hepática é um processo complexo envolvendo células parenquimatosas e não parenquimatosas residentes no fígado, assim como o recrutamento de outros tipos celulares em resposta ao dano e a inflamação (GRESSNER & BACHEN, 1995). A progressão do dano hepático é caracterizada, inicialmente, pela esteatose, seguida de inflamação, necrose e apoptose, fibrose e em alguns indivíduos, pela fibrose (TILG & DIEHL, 2000).

O consumo do álcool regula de forma direta ou indireta o metabolismo lipídico por aumentar a expressão de genes envolvidos na síntese de ácidos graxos e suprimir genes relacionados com a oxidação lipídica, resultando em esteatose hepática (MANDREKAR, 2007). A regulação da síntese e oxidação de ácidos graxos é complexa, envolvendo múltiplos sistemas enzimáticos em diferentes locais dos hepatócitos. Entretanto, a expressão de muitos genes envolvidos no metabolismo de ácidos graxos é coordenada e responde a estímulos de fatores de transcrição específicos (SMITH, 2002).

Um desses fatores de transcrição é o receptor ativado por proliferadores de peroxissomos alfa (PPAR-α), um receptor nuclear hormonal que controla a transcrição de genes envolvidos no transporte e oxidação dos ácidos graxos (YU *et al*., 2003; WAGNER *et al*., 2011). O acetaldeído, metabólito do etanol, inibi diretamente a ativação transcricional e a habilidade de ligação ao DNA do PPARα nos hepatócitos (GALLI *et al*., 2001). O alcoolismo também pode, indiretamente, inibir o PPARα por aumentar a regulação do citocromo P450 2E1 derivado do estresse oxidativo (LU *et al*., 2008) ou por diminuir a regulação de adiponectina e zinco, que ativam o PPARα (YOU *et al*., 2005; KANG *et al*., 2009).

Além de atuar no PPARα o metabolismo do etanol também pode ativar o receptor ativado por proliferadores de peroxissomos gama (PPARγ). O PPARγ é expresso em macrófagos e está implicado na resposta imune inata (DAYNES & JONES, 2002). Sua expressão está aumentada durante o desenvolvimento da esteatose

hepática em resposta a dieta com alto teor lipídico, obesidade e exposição crônica ao etanol (VIDAL-PUIG *et al*., 1996; MEMON *et al*., 2000; RAHIMIAN *et al*., 2001; BOELSTERLI & BEDOUCHA, 2002;). Durante a exposição ao álcool, a expressão do PPARγ foi observada nas células de Kupffer e nos hepatócitos (BOELSTERLI & BEDOUCHA, 2002) e o tratamento com agonista do PPARγ preveniu o desenvolvimento da esteatose e da inflamação (ENOMOTO *et al*., 2003).

OBJETIVO:

Avaliar os efeitos do tratamento da DEC em modelo de alcoolismo crônico na homeostase lipídica através de marcadores como o MDA, PPARα e por dosagem hepática de triglicerídeos, assim como, analisar a ativação de outros fatores de transcrição como o PPARγ.

RESULTADOS PRELIMINARES

Efeitos da DEC na homeostase lipídica

O Malondialdeído (MDA) é um produto da peroxidação lipídica que atua na modulação da resposta inflamatória hepática. O consumo de álcool produz danos hepáticos graves que são indicados pelo aumento de MDA. A himunohistoquímica mostrou uma grande marcação de MDA no grupo alcoólico (Fig. 1A). No grupo EtOH50 esse marcador apresenta-se significativamente diminuído (Fig. 1B).

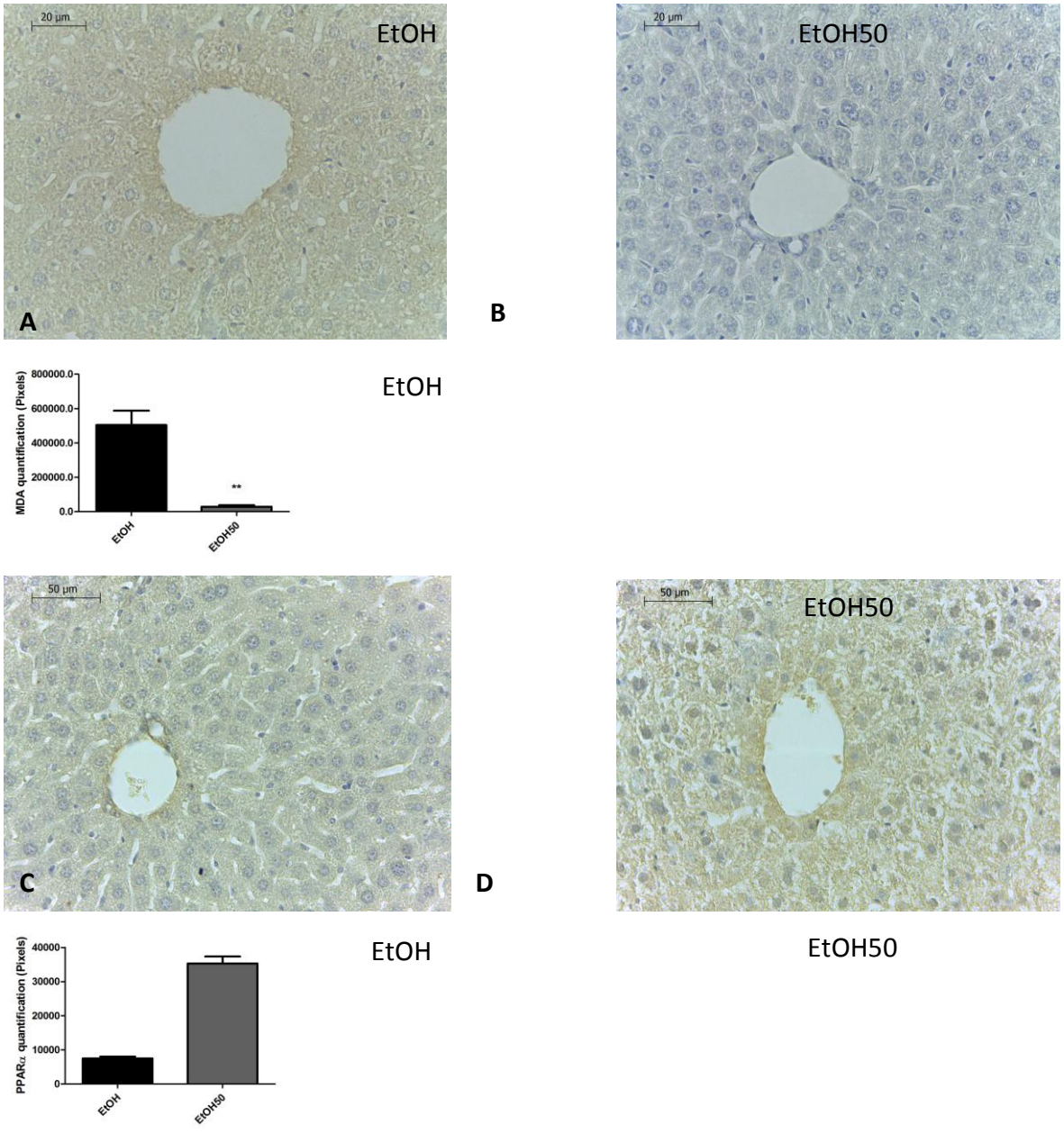
O consumo do álcool também inibe a oxidação de ácidos graxos nos hepatócitos via inativação do PPAR-α. O grupo EtOH mostrou uma diminuição da expressão de PPAR-α (Fig. 1C e 2A). Após o tratamento com DEC (EtOH50), observou-se um aumento significativo da expressão de PPAR-α (Fig. 1D e Fig. 2A).

Efeitos da DEC na ativação do PPAR-γ

A expressão do PPAR-γ está aumentada durante o desenvolvimento da esteatose hepática. O consumo de etanol aumentou expressão de PPAR-γ (Fig. 1E e 2B). Após o

tratamento com DEC observou-se uma diminuição significativa da expressão desse fator de transcrição (Fig. 1F e 2B).

FIGURA 1



EtOH

EtOH50

*

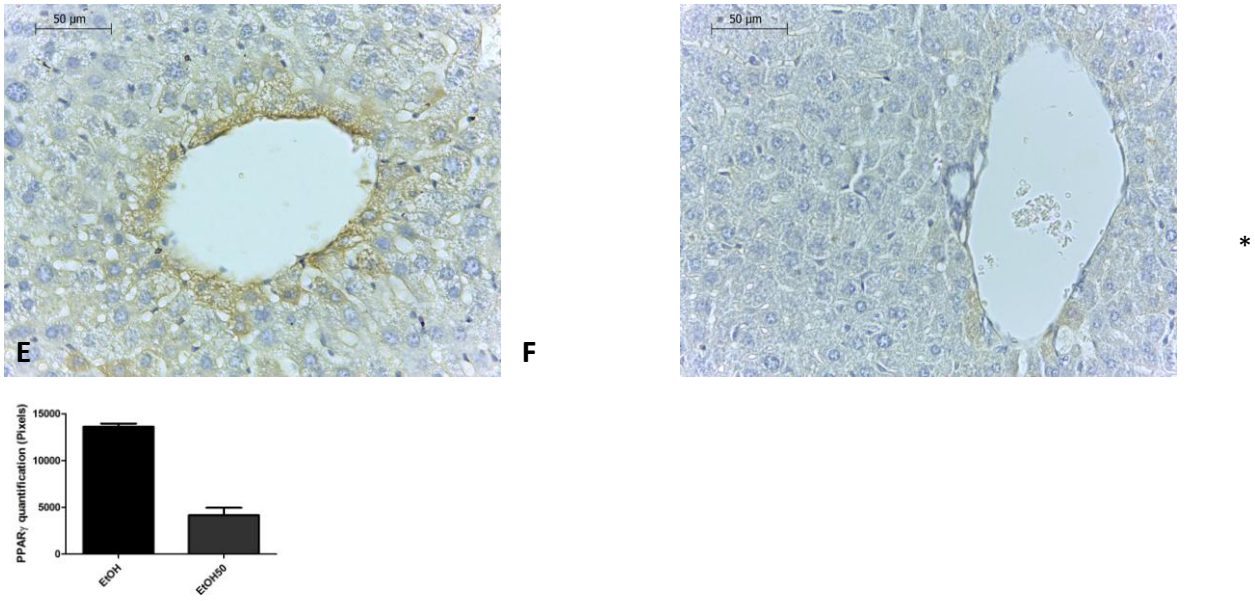


Figura 1. Imunohistoquímica do fígado. Grupo alcoólico (EtOH): **(A)** MDA, **(C)** PPARα e **(E)** PPARγ. Grupo alcoólico tratado com DEC (EtOH50): **(B)** MDA, **(D)** PPARα e **(F)** PPARγ. Intensa marcação nos hepatócitos para MDA e PPARγ no grupo EtOH. Após o tratamento com DEC foi observada uma redução da marcação. O grupo EtOH apresentou baixa expressão de PPARα, enquanto os animais tratados com DEC apresentaram uma alta expressão desse receptor. n= 5 animais para cada grupo. **P* < 0.05, ***P*<0.01.

FIGURA 2

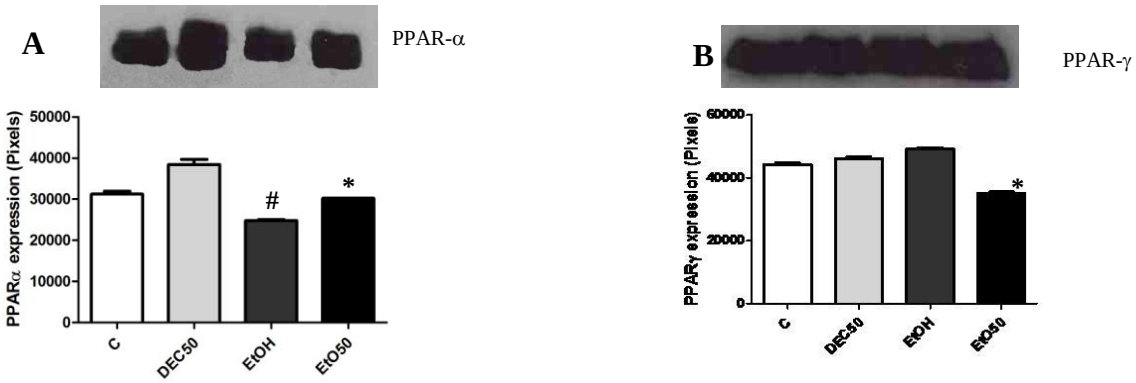


Fig 2. Westen blot. Os extratos hepáticos foram utilizados para analisar a expressão de PPAR-α **(A)** e PPAR-γ **(B)**. O consumo de etanol aumentou a expressão de PPAR-γ e diminuiu a expressão de PPAR-α. # p <0.05, grupo EtOH comparado com o grupo controle. * p <0.05, grupo EtOH50 comparado com o grupo EtOH. Os resultados foram obtidos de ensaio em triplicata (n= 4 animais para cada grupo).

CONCLUSÃO PRELIMINAR

Estes resultados sugerem que possivelmente a DEC diminui o desenvolvimento da esteatose e da inflamação hepática por atuar em fatores de transcrição específicos como o PPARα e o PPARγ que controlam o metabolismo de ácidos graxos e a resposta imune inata.

REFERÊNCIAS

BOELSTERLI, U.A.; BEDOUCHA, M. Toxicological consequences of altered peroxisome proliferator-activated receptor γ (PPARγ) expression in the liver: insights from models of obesity and type 2 diabetes. **Biochem Pharmacol,** 63:1–10, 2002.

DAYNES, R. A.; JONES, D. C. Emerging roles of PPARs in inflammation and immunity. **Nat Rev Immunol,** 2:748–59, 2002.

ENOMOTO, N.; TAKEI, Y.; HIROSE, M.; KONNO, A.; SHIBUYA, T.; MATSUYAMA, S., SUZUKI, S.; KITAMURA, K.I.; SATO, N. Prevention of ethanol-induced liver injury in rats by an agonist of peroxisome proliferator-activated receptor-gamma, pioglitazone. **J Pharmacol Exp Ther,** 306: 846-854, 2003.

GALLI, A.; PINAIRE, J.; FISCHER, M.; DORRIS, R.; CRABB, D.W. The transcriptional and DNA binding activity of peroxisome proliferator-activated receptor alpha is inhibited by ethanol metabolism. A novel mechanism for the development of ethanol-induced fatty liver. **J Biol Chem,** 276: 68-75, 2001.

GRESSNER, A.M, BACHEM, M. GMolecular mechanisms of liver fibrogenesis—a homage to the role of activated fat-storing cells. **Digestion,** 56:335–46, 1995.

KANG, X.; ZHONG, W.; LIU, J.; et al. Zinc supplementation reverses alcohol-induced steatosis in mice through reactivating hepatocyte nuclear factor-4alpha and peroxisome proliferator-activated receptor-alpha. **Hepatology,** 50:1241–1250, 2009.

LU, Y.; ZHUGE, J.; WANG, X.; et al. Cytochrome P450 2E1 contributes to ethanol-induced fatty liver in mice. **Hepatology,** 47: 1483–1494,2008.

MANDREKAR, P. Signaling mechanisms in alcoholic liver injury: Role of transcription factors, kinases and heat shock proteins. **World J Gastroenterol,** 13: 4979-4985, 2007.

MEMON, R.A.; TECOTT, L. H.; NONOGAKI, K.; BEIGNEUX, A.; MOSER, A. H.; et al. Up-regulation of peroxiso me proliferatoractivated receptors (PPAR-α) and PPAR-γ messenger ribonucleic acid expression in the liver in murine obesity: troglitazone induces expression of PPAR-γ -responsive adipose tissue-specific genes in the liver of obese diabetic mice. **Endocrinology**, 141:4021–31, 2000.

NAGY, L.E. Molecular aspects of alcohol metabolism: Transcription factors involved in early ethanol-induced liver injury. **Annu Rev Nutr,** 24: 55-78, 2004.

RAHIMIAN, R.; MASIH-KHAN, E.; LO, M.; VAN BREEMEN, C.; MCMANUS, B. M.; DUBE, G. P. Hepatic over-expression of peroxisome proliferator activated receptor γ 2 in the ob/ob mouse model of non-insulin dependent diabetes mellitus. **Mol Cell Biochem**, 224:29–37, 2001.

SMITH, S.A. Peroxisome proliferatoractivated receptors and the regulation of mammalian lipid metabolism. **Biochem Soc Trans**, 30:1086–90, 2002.

TILG, H.; DIEHL, A.M. Cytokines in alcoholic and nonalcoholic steatohepatitis. **N Engl J Med,** 343: 1467–76, 2000.

VIDAL-PUIG, A., JIMENEZ-LINAN, M.; LOWELL, B. B.; HAMANN, A.; HU, E.; et al. Regulation of PPARγ gene expression by nutrition and obesity in rodents. **J Clin Invest**, 97:2553–61, 1996.

WAGNER, M.; ZOLLNER, G.; TRAUNER, M. Nuclear receptors in liver disease. **Hepatology**, 53:1023–1034, 2011.

YOU, M.; CONSIDINE, R. V.; LEONE, T.C.; et al. Role of adiponectin in the protective action of dietary saturated fat against alcoholic fatty liver in mice. **Hepatology,** 42:568–577, 2005.

YU, S., RAO, S.; REDDY, J.K. Peroxisome proliferator-activated receptors, fatty acid oxidation, steatohepatitis and hepatocarcinogenesis. **Curr Mol Med,** 3:561–572, 2003.

CONCLUSÃO

No presente estudo a DEC se mostrou altamente efetiva na prevenção da ALD experimental. Além de prevenir o dano hepático induzido pelo álcool, a DEC reduziu a peroxidação lipídica e a ativação do NF-κB por estabilizar a degradação da IκBα e, também, por reduzir a expressão de citocinas pro-inflamatórias, iNOS e COX-2.

ANEXOS

ANEXO A: PARECER DO COMITÊ DE ÉTICA

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Certificamos que o protocolo (P-04/10.4), intitulado **"Efeito da Dietilcarbamazina (DEC) sobre Hepatócitos de Camundongos Normais, Desnutridos e Expostos ao Etanol"**, sob a responsabilidade de **SURA WANESSA SANTOS ROCHA,** atende ao disposto na Lei 11794/08, que dispõe sobre o uso científico no uso de animais, inclusive, aos princípios da Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL). A referida licença não exime a observância das Leis e demais exigências legais na vasta legislação nacional.

Esta licença tem validade até 13/09/2014 e inclui o uso total de:

Mus musculus

- 96 machos de C57 BL6/J, idade: 20 dias.

Rio de Janeiro, 04 de outubro de 2010.

Dr. Zilton F. M. de Vasconcelos
Coordenador em Exercício
CEUA/FIOCRUZ

Comissão de Ética no Uso de Animais
Vice-Presidência de Pesquisa e Laboratórios de Referência - Fundação Oswaldo Cruz
Av. Brasil, 4036 – Prédio da Expansão – sala 200 – Manguinhos – Rio de Janeiro / RJ
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ANEXO B: COMPROVAÇÃO DE SUBMISSÃO DO ARTIGO 1

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Manuscript Number:

Title: Diethylcarbamazine prevents alcohol-induced liver injury in C57BL/6 mice by inhibiting the NF-κB activation

Article Type: Research Paper

Section/Category: Pulmonary, gastrointestinal and urogenital pharmacology

Keywords: Diethylcarbamazine, Alcoholism, Hepatic injury, Inflammatory markers, transcription factors, NF-κB

Corresponding Author: Mrs. bruna silva,

Corresponding Author's Institution: Centro de Pesquisas Aggeu Magalhães

First Author: Bruna S Silva

Order of Authors: Bruna S Silva; Gabriel B Rodrigues; Sura Wanessa S Rocha; Edlene L Ribeiro; Fabiana O Gomes; Amanda Karolina S Silva; Christina A Peixoto

Abstract: Induction of NF-κB-mediated gene expression has been identified in the pathogenesis of alcoholic liver disease ALD). Diethylcarbamazine (DEC) is a piperazine derivative drug with anti-inflammatory properties. The present study was designed to evaluate the effect of DEC on NF-κB pathways undergoing alcoholism induced hepatic inflammation. Forty male C57BL/6 mice were divided equally into four groups: control group (C); DEC- treated group, which received 50 mg/kg (DEC50); the alcoholic group (EtOH), submitted to chronic alcohol consumption and the alcohol-DEC treated group (EtOH50), submitted to chronic alcoholism consumption plus DEC treatment. Histological analysis of the alcoholic group showed evident hepatocellular damage which was reduced in the EtOH50 group. Immunohistochemistry and western blot results showed elevated expression of inflammatory markers such as MDA, TNF-α, IL-1β, COX-2 and iNOS in hepatocytes of the EtOH group. However, low immunopositivity for these markers was detected following DEC treatment. In the EtOH group the activation of NF-κB was observed by an increase in the expression of both NF-κB and pNF-κB in hepatocytes. This expression was significantly reduced in the EtOH50 livers. Protein expression of IκBα was measured to determine whether activation of NF-κB might be the result of IκBα degradation. It was observed that expression of this enzyme was low in the EtOH group, while animals treated with DEC had a high expression of IκBα. The results of the present study indicate that DEC alleviates alcoholic liver injury, in part by the inhibiting activation of NF-κB and by suppressing the induction of NF-κB-dependent genes.

ANEXO C: NORMAS DA REVISTA ARTIGO 1

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Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Please make sure to limit the length of this paragraph to max. 500 words.

Material and methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. Avoid extensive citations and discussion of published literature. Please make sure to limit the length of this paragraph to max. 1500 words.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion section.

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• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that phone numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.**
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A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

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A Graphical abstract is optional for this journal. It should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more, but should be readable on screen at a size of 200 × 500 pixels (at 96 dpi this corresponds to 5 × 13 cm). Bear in mind readability after reduction, especially if using one of the figures from the article itself. Preferred file types: TIFF, EPS, PDF or MS Office files. See http://www.elsevier.com/graphicalabstracts for examples.

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Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and nomenclature, and avoiding general and plural terms and multiple concepts (avoid, for example, "and", "of"). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations
Abbreviations are a hindrance for the reader. Use as few abbreviations as possible and write out names of compounds, receptors, etc., in full throughout the text of the manuscript, with the exceptions given HERE.

Unnecessary abbreviations. Unnecessary abbreviations such as AP, TEP, TFT, CER, nAc and LTFSE (for afterpotential, transepithelial potential, Tail-flick test, cold-exposed rats, nucleus accumbens and sympatho-excitatory lateral tegmental field) are **not** acceptable.

Receptor abbreviations. Receptor abbreviations such as βAR, mAChR, BZR for β-adrenoceptor, muscarinic receptor, benzodiazepine receptor, respectively, should **not** be used. For receptors avoid the abbreviation "R". With receptor subtypes mention the full receptor name throughout the manuscript e.g., adenosine A1 receptor, dopamine D2 receptor, melanocortin MC3 receptor, endothelin ETA receptor.
Generic names. Generic names should **not** be abbreviated. For example, AMP, HAL, HIST, RAMH, TAM, SST, for amphetamine, haloperidol, histamine, (R)-a -methylhistamine, tamoxifen, somatostatin, are not accepted. Abbreviations such as (e.g., NA, DA, ACh, ET for noradrenaline, dopamine, acetylcholine, endothelin, should **not** be used.
Abbreviations which have come to replace the full term. Abbreviations which have come to replace the full term (e.g., GABA, DOPA, EDRF, 5HT, for γ-aminobutyric acid, 3,4-dihydroxyphenylalanine, endothelium-derived relaxing factor, 5-hydroxytryptamine) may be used, provided the term is spelled out in the abstract and in the body of the manuscript the first time the abbreviation is used.
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Authors not conforming to these demands will have their manuscripts returned for correction, with delayed publication as the result.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

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Only generic and chemical names of drugs should be used, although a proprietary equivalent may be indicated once, in parentheses. The nomenclature of chemical substances should be consistent, clear and unambiguous, and should conform to the usage of the American Chemical Society and the convention recommended by the International Union of Pure and Applied Chemistry (IUPAC, http://www.iupac.org/general/FAQs/ns.html) When in doubt, writers should consult the indexes of Chemical Abstracts; the various reports and pamphlets of the American Chemical Society Committee on Nomenclature, Spelling and Pronunciation; the recommendations of the IUBMB (http://www.chem.qmul.ac.uk/iubmb) When drugs which are mixtures of stereoisomers are used, the fact that they have a composite nature and the implication of this for interpretation of the data and drawing of conclusions should be made clear. The use of the appropriate prefix is essential. Use of the generic name alone without a prefix would be taken to refer to agents with no stereoisomers. The nomenclature of receptors and their subtypes and of ion channels should conform to NCIUPHAR (http://www.iuphar.org/nciuphar.html) he trivial name of enzymes may be used in the text, but the systematic name and classification number according to Enzyme Nomenclature of the NC-IUBMB (http://www.chem.qmul.ac.uk/iubmb/enzyme/), rev. edn. (Academic Press, New York, NY, 1984) should be quoted the first time an enzyme is mentioned.

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Sample references *Periodicals*:

Barnes, P.J., Karin, M., 1997. Nuclear factor-k β -a pivotal transcription factor in chronic inflammatory diseases. N. Engl. J. Med 336, 1066 -1071. Paivio, A., Jansen, B., Becker, L.J., 1975. Comparisons through the mind's eye. Cognition 37, 635-647.

Books:

Strunk, W., White, E.B., 1979. The Elements of Style, third ed. Macmillan, New York, NY. Gurman, A.S., Kniskern, D.P., 1981. Family therapy outcome research: knowns and unknowns. In: Gurman, A.S., Kniskern, D.P. (Eds.), Handbook of Family Therapy. Brunner/Maazel, New York, NY, pp. 742-775.

Order of references:
De Groat, W., 1990.
Maggi, C.A., 1988.
Maggi, C.A., Lecci, A., 1987
Maggi, C.A., Meli, A., 1986
Maggi, C.A., Santicoli, P., Meli, A., 1984.
Magi, C.A., Giuliani, S., Patacchini, R., Rovero, P., Giachetti, A., Meli, A., 1989a.
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All necessary files have been uploaded, and contain:
- Keywords
- All figure captions
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ANEXO D: COMPROVAÇÃO SUBMISSÃO ARTIGO 2

Drugs

ANTI-INFLAMMATORY EFFECTS OF DIETHYLCARBAMAZINE: A REVIEW

--Manuscript Draft--

Manuscript Number:	
Full Title:	ANTI-INFLAMMATORY EFFECTS OF DIETHYLCARBAMAZINE: A REVIEW
Article Type:	Review Article
Abstract:	Diethylcarbamazine (DEC) is a well-known drug used throughout the world against lymphatic filariasis. However, along the last years, innumerous studies indicated other impressive pharmacological activities of DEC. Some preliminary clinical studies have stated that DEC was quite effective in asthmatic conditions, and various experimental studies used DEC as a potent leukotriene inhibitor. Nowadays, is well-established that DEC interferes with the cyclooxygenase pathways, reducing thromboxane, prostacyclin and prostaglandin production. Recent studies using different experimental models of inflammation indicated that DEC, besides inhibiting cyclooxygenases and lipoxygenases pathways, can also inhibit the nuclear transcription factor kappa B (NF-kB) activation, which is a key regulator of proinflammatory genes such us TNF-α, IL-1β, nitric oxide synthase inducible (iNOS) and even cyclooxygenase 2 (COX-2). This review will provide a comprehensive summary of DEC, including information on filaricidal action, inhibition of synthesis and secretory pathways, immunomodulatory activities, and specific inhibition of lipoxygenase and cyclooxygenase pathways.
Corresponding Author:	Christina Alves Peixoto, PhD Fundação Oswaldo Cruz Recife, Pernambuco BRAZIL
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Fundação Oswaldo Cruz
Corresponding Author's Secondary Institution:	
First Author:	Christina Alves Peixoto, PhD
First Author Secondary Information:	
Order of Authors:	Christina Alves Peixoto, PhD
	Bruna Santos Silva, Ms
Order of Authors Secondary Information:	
Author Comments:	Dear Editor, We are pleased to submit to the Drugs journal the manuscript entitled ANTI-INFLAMMATORY EFFECTS OF DIETHYLCARBAMAZINE: A REVIEW. Diethylcarbamazine (DEC) has been used successfully as a principal public health tool to eliminate lymphatic filariasis in several countries throughout the world, however its mechanism of action remains controversial. Pharmacological studies have showed that DEC interferes with the arachidonic acid metabolism, acting as an anti-inflammatory drug. There is substantial information to suggest that DEC blocks a number of steps in both cyclooxigenase (COX) and lipoxigenase pathways, including inhibition of leucocyte chemotaxis, granulocyte degranulation, and peripheral vasodilation. Recently, some studies demonstrated that DEC can also inhibit the NF-κB activation. This review will provide a comprehensive summary of DEC, including information on filaricidal action, inhibition of synthesis and secretory pathways, immunomodulatory activities, and specific inhibition of lipoxygenase and cyclooxygenase pathways. Sincerely, Christina Peixoto

ANEXO E: NORMAS DA REVISTA ARTIGO 2

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| Drugs – incl. option to publish open access

Drugs

Editor: D. Peters

ISSN: 0012-6667 (print version)
ISSN: 1179-1950 (electronic version)

Journal no. 40265

Instructions for Authors

Instructions for Authors

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on the clinical aspects of drug treatment, and those with a strong link to clinical practice.

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The title page should include:

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Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

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CONSORT statement

PRISMA statement

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- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

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LaTeX macro package (zip, 182 kB)

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Abbreviations should be defined at first mention and used consistently thereafter.

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reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

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Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

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Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3, 7].

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- Article by DOI

  Slifka MK, Whitton JL. Clinical implications of dysregulated cytokine production. J Mol Med. 2000; doi:10.1007/s001090000086

- Book

  Blenkinsopp A, Paxton P. Symptoms in the pharmacy: a guide to the management of common illness. 3rd ed. Oxford: Blackwell Science; 1998.

- Book chapter

  Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. In: Bourne GH, Danielli JF, Jeon KW, editors. International review of cytology. London: Academic; 1980. pp. 251–306.

- Online document

  Doe J. Title of subordinate document. In: The dictionary of substances and their effects. Royal Society of Chemistry. 1999. http://www.rsc.org/dose/title of subordinate document. Accessed 15 Jan 1999.

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- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

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For the best quality final product, it is highly recommended that you submit all of your artwork – photographs, line drawings, etc. – in an electronic format. Your art will then be produced to the highest standards with the greatest accuracy to detail. The published work will directly reflect the quality of the artwork provided.

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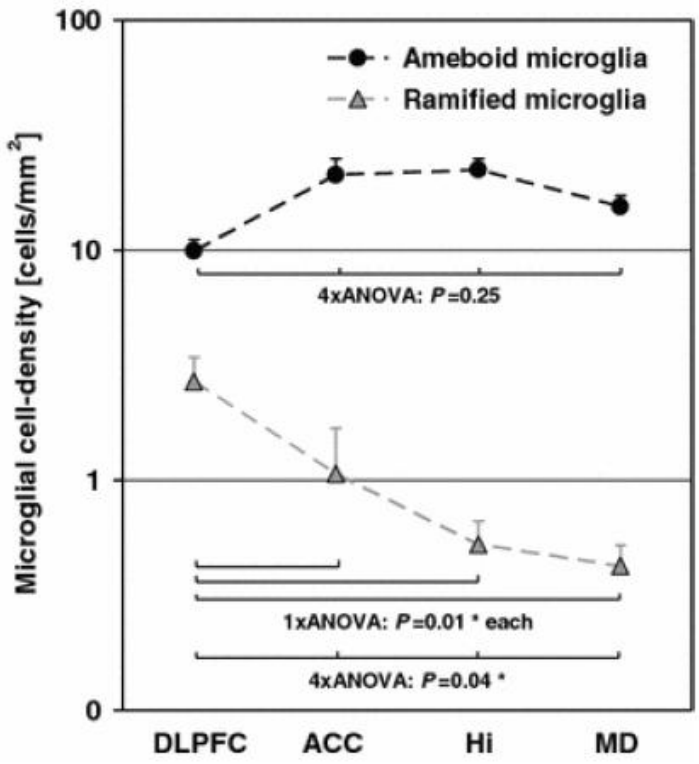
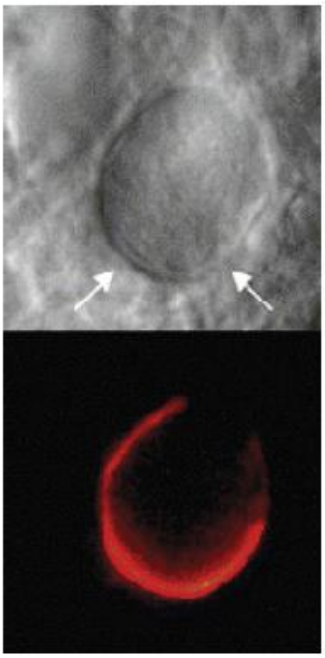
Line Art



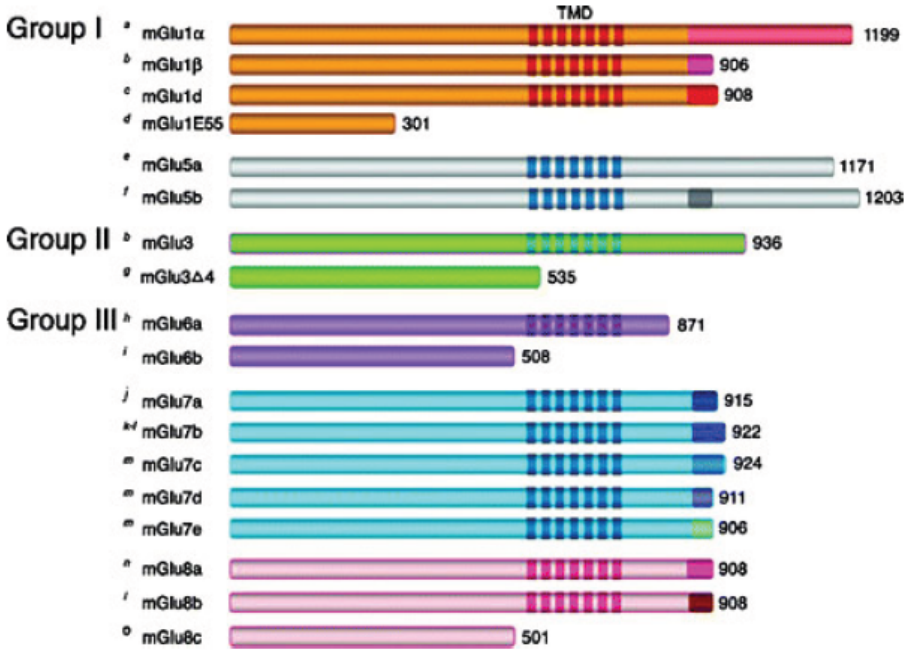
- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art

- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
- Halftones should have a minimum resolution of 300 dpi.



Combination Art



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- Combination artwork should have a minimum resolution of 600 dpi.

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P/ Maria Teresa Jansen

Prof. Abel Vieira Neto
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ANEXO F: ARTIGOS PUBLICADOS EM PERIÓDICOS NO PERÍODO 2009-20013

1. Rocha, S.W.S, **SILVA, B. S.**, GOMES, F. O. S., Silva, AKS, Raposo, C, BARBOSA, K. P. S., Cipriano, D.O., Santos, AC. O., PEIXOTO, C. A.
Effect of diethylcarbamazine on chronic hepatic inflammation induced by alcohol in C57BL/6 mice. European Journal of Pharmacology. , v.689, p.194 - 203, 2012.

2. Rocha, S.W.S, Santos, AC. O., **SILVA, B. S.**, Cipriano, D.O., Ribeiro, E.L., BARBOSA, K. P. S., GOMES, F. O. S., PEIXOTO, C. A.
Effects of Diethylcarbamazine (DEC) on Hepatocytes of C57BL/6J Mice Submitted to Protein Malnutrition. Journal of Food and Drug Analysis. , v.20, p.524 - 531, 2012.

3. Silva, AKS, Cipriano, D.O., Rocha, S.W.S, GOMES, F. O. S., **Silva, B. S.**, Santos, AC. O., LIMA, M. C. A., GALDINO, S. L., PITTA, I. R., SOUZA, José Roberto de, PEIXOTO, C. A.
Effect of New Thiazolidine Derivatives LPSF/GQ-02 and LPSF/GQ-16 on Atherosclerotic Lesions in LDL receptor-deficient mice (LDLR-/-). Cardiovascular Pathology. , 2012.

4. de Melo, F. M., Vasconcelos, L. R. S., do Carmo, R. F., **Silva, B. S.**, MOURA, P., Cavalcanti, M. doS. deM., Pereira, L. M. M. B., Lacerda, H. R.
MBL2 polymorphism and autoimmune markers: reconsidering the complexity of biological systems in the choice of controls. International Journal of Immunogenetics. , v.38, p.105 - 108, 2011.

5. Melo, F. M., Vasconcelos, L. R. S., SILVA, B. S., MOURA, P., CAVALCANTI, M. S. M., Pereira, L. M. M. B., Lacerda, H. R.
Structural polymorphism of the mannose-binding lectin 2 (MBL2) gene in HCV-infected patients with a serological marker for thyroid autoimmunity. International Journal of Immunogenetics. , v.36, p.377 - 381, 2009.