UNIVERSIDADE FEDERAL DO PARÁ

INSTITUTO DE CIÊNCIAS DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

EFEITO DO METABOLISMO DO ETANOL NO PADRÃO *BINGE* DA ADOLESCÊNCIA À VIDA ADULTA E SUAS REPERCUSSÕES HEPÁTICAS

Thais Pereira Torres Magno

Belém-PA

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RESUMO

TORRES-MAGNO, T.P. - **Efeito do metabolismo do etanol no padrão *binge* da adolescência à vida adulta e suas repercussões hepáticas -** Dissertação (Mestrado) - Programa de Pós-Graduação em Ciências Farmacêuticas – Universidade Federal do Pará, Belém, 2024

O padrão de consumo intermitente e episódico eleva a concentração de álcool no sangue a níveis perigosos (≥ 0,08 g/dL), provocando desequilíbrios homeostáticos, especialmente no fígado, principal órgão responsável pela metabolização do etanol (EtOH). Durante a biotransoformação do EtOH há a formação de espécies reativas de oxigênio (ROS) e subprodutos tóxicos, como acetaldeído, que corroboram para o desenvolvimento de lesões hepáticas. O consumo excessivo de álcool forma episódica, denominada em *binge drinking* (BD) pode progredir de um quadro de esteatose para esteato-hepatite, com o surgimento de inflamação e necrose. Apesar dos extensos achados sobre as repercussões hepáticas do BD, ainda não foram investigadas quais impactos de administrações repetidas de álcool da adolescência à fase adulta induzem alterações no fígado. Diante disso, o presente estudo teve como objetivos realizar um estudo bibliométrico da produção científica sobre o consumo intermitente e episódico de EtOH e suas implicações hepáticas, seguido por uma investigação experimental dos efeitos desse consumo a curto e longo prazo em ratas administradas da adolescência à fase adulta. No estudo bibliométrico foram selecionados os 100 artigos mais citados sobre o consumo de EtOH em padrão *binge* e hepatotoxicidade. Esses documentos foram recuperados na base de dados *Web of Science Core Collection* (WoS-CC) e tiveram seus dados bibliométricos extraídos. Para o estudo experimental, foram utilizadas 62 ratas Wistar fêmeas, submetidas ao protocolo de BD, que consistiu em três dias consecutivos de administração intragástrica de EtOH (3,0 g/kg/dia; 20% v/v) ou água destilada, seguidos por quatro dias de abstinência, durante oito semanas (oito ciclos). Após a administração, foram coletadas amostras de soro e fígado para análises histopatológicas e bioquímicas do perfil lipídico e oxidativo, com avaliação dos efeitos a curto prazo (24 horas) e a longo prazo (14 dias). O mapeamento do conhecimento revelou que o consumo de álcool em padrões *binge* pode causar diversos tipos de danos ao fígado, incluindo esteatose, inflamação, estresse oxidativo, apoptose de hepatócitos, disfunção mitocondrial, ativação de células estreladas hepáticas e alterações no metabolismo lipídico. É importante destacar que a intensidade e a manifestação desses danos podem variar de acordo com o protocolo de BD aplicado, considerando fatores como a dose, a frequência e a duração da exposição ao EtOH. Nesse contexto, o estudo experimental complementa os achados do

estudo bibliométrico ao fornecer evidências sobre os impactos de repetidos ciclos de BD no fígado em curto e em longo prazo. O estudo experimental revelou aumento nos marcadores de transaminases hepáticas e no metabolismo lipídico (triglicerídeos e VLDL), com níveis elevados tanto em curto quanto em longo prazo. A análise histológica confirmou o efeito hepatotóxico do álcool, evidenciando esteatose (grau 2), necrose e fibrose nos animais expostos. Houve também um aumento significativo na peroxidação lipídica após 8 ciclos de BD, que permaneceu elevado após 14 dias de abstinência, além de alterações prolongadas nas atividades das enzimas superóxido dismutase e catalase. Esses achados destacam a necessidade de aprofundar o conhecimento sobre os mecanismos da hepatotoxicidade induzida pelo BD, especialmente durante a adolescência, uma fase crítica para o desenvolvimento de doenças hepáticas na vida adulta.

Palavras-Chave: Adolescente, *binge drinking*; doença hepática alcoólica; fígado

ABSTRACT

TORRES-MAGNO, T.P. - **Effect of ethanol metabolism on the binge pattern from adolescence to adulthood and its hepatic repercussions** - Dissertation (Master's Degree) - Postgraduate Program in Pharmaceutical Sciences - Federal University of Pará, Belém, 2024

The pattern of intermittent and episodic consumption raises the alcohol concentration in the blood to dangerous levels (≥ 0.08 g/dL), causing homeostatic imbalances, especially in the liver, the main organ responsible for metabolizing ethanol (EtOH). During the biotransformation of EtOH, reactive oxygen species (ROS) and toxic by-products such as acetaldehyde are formed, which contribute to the development of liver damage. Episodic binge drinking (BD) can progress from steatosis to steatohepatitis, with the appearance of inflammation and necrosis. Despite the extensive findings on the hepatic repercussions of BD, the impact of repeated administrations of alcohol from adolescence to adulthood on changes in the liver has not yet been investigated. In view of this, the present study aimed to carry out a bibliometric study of scientific production on intermittent and episodic consumption of EtOH and its hepatic implications, followed by an experimental investigation of the effects of this consumption in the short and long term in rats administered from adolescence to adulthood. The bibliometric study selected the 100 most cited articles on binge drinking and hepatotoxicity. These documents were retrieved from the Web of Science Core Collection (WoS-CC) database and their bibliometric data extracted. For the experimental study, 62 female Wistar rats were subjected to the BD protocol, which consisted of three consecutive days of intragastric administration of EtOH (3.0 g/kg/day; 20% v/v) or distilled water, followed by four days of abstinence, for eight weeks (eight cycles). After administration, serum and liver samples were collected for histopathological and biochemical analyses of the lipid and oxidative profile, short-term (24 hours) and long-term (14 days) effects were assessed. The mapping of knowledge revealed that the consumption of binge drinking can cause various types of liver damage, including steatosis, inflammation, oxidative stress, hepatocyte apoptosis, mitochondrial dysfunction, activation of hepatic stellate cells and changes in lipid metabolism. It is important to note that the intensity and manifestation of this damage may vary according to the BD protocol applied, taking into account factors such as the dose, frequency and duration of exposure to EtOH. In this context, the experimental study complements the findings of the bibliometric study by providing evidence on the impacts of repeated cycles of BD on the liver in the short and long term. The experimental study revealed an increase in liver transaminase markers and lipid metabolism (triglycerides and VLDL), with elevated levels in both the short and long term.

Histological analysis confirmed the hepatotoxic effect of alcohol, showing steatosis (grade 2), necrosis and fibrosis in the exposed animals. There was also a significant increase in lipid peroxidation after 8 cycles of BD, which remained high after 14 days of abstinence, as well as prolonged changes in the activities of the enzymes superoxide dismutase and catalase. These findings highlight the need to deepen knowledge about the mechanisms of BD-induced hepatotoxicity, especially during adolescence, a critical phase for the development of liver disease stage for the development of liver disease in adulthood.

Keywords: Adolescent, binge drinking; alcoholic liver disease; liver

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LISTA DE ABREVIATURAS E SIGLAS

ADH Álcool Desidrogenase

ALDH Aldeído Desidrogenase

ALT Aspartato aminotransferase

AST Alanina aminotransferase

CAT catalase

MEOS do ingês *Ethanol-oxidizing system*

NAD Nicotinamida-adenina-dinucleotídeo

NADPH Nicotina-Adenina-Dinucletídio-Fosfoto Hidrogenado

NO Óxido nítrico

OMS Organização Mundial da Saúde

EtOH Etanol

NIAAA Instituto Nacional de Abuso de Álcool e Alcoolismo

CISA Centro de Informações sobre Saúde e Álcool

INPAD Instituto Nacional de Políticas Públicas do Álcool e Outras Drogas

CYP2E1 citocromo P450 CYP2E1, subfamília E, membro 1

FAS do inglês *Fas cell surface death receptor*

H2O fórmula molecular da água

DNA do inglês *deoxyribonucleic acid*

CO2 fórmula molecular do dióxido de carbono

TNF do inglês *tumor necrosis factor*

IL do inglês interleukin

TGF do inglês *transforming growth factor*

BD do inglês *binge drinking*

Sumário

Sumário

1. Introdução

1.1. Considerações gerais

O etanol (EtOH), também conhecido como álcool etílico, é uma droga psicodepressora com efeito sedativo-hipnótico, amplamente consumida em todo o mundo (Hoffmann, 1996; Morean et al., 2019). Seu uso está enraizado em práticas culturais há séculos, o que contribui para sua aceitação social (OMS, 2018). Sendo uma substância lícita na maioria dos países, de fácil acesso e frequentemente associada ao prazer, tanto de forma explícita quanto implícita, seu consumo elevado é incentivado, especialmente entre jovens e adolescentes (Heim et al., 2008).

O uso de álcool está associado a diversos problemas sociais, morais e de saúde (Heim et al., 2018). As consequências desse consumo excessivo têm sido foco de interesse público e científico, pois os prejuízos envolvem desde a queda na produtividade econômica e o impacto no sistema de justiça criminal até sobrecargas no sistema de saúde e outras instituições sociais (Beaglehole et al., 2009; OMS, 2018).

O consumo excessivo de álcool tem se tornado uma preocupação crescente, especialmente entre jovens e adolescentes, uma vez que nos últimos anos tem-se observado um início precoce no consumo de bebidas alcoólicas (Sayette, 2017; Morean et al., 2019). Segundo o Instituto Nacional de Abuso de Álcool e Alcoolismo (NIAAA), adolescentes que começam a consumir EtOH antes dos 15 anos têm um risco quatro vezes maior de desenvolver dependência à substância. Apesar desses dados alarmantes, estima-se que em diversos países das Américas e da Europa, 26,5% dos adolescentes iniciem o consumo de álcool antes dos 15 anos (NIAAA, 2017; OMS, 2018).

A literatura científica apresenta uma série de evidências sobre os danos à saúde associados ao consumo excessivo de álcool. Segundo a OMS (2018), o EtOH está relacionado a mais de 200 doenças e condições de saúde, incluindo desmaios, intoxicações, lesões, acidentes de trânsito, mortes, agressões sexuais ou físicas, infecções sexualmente transmissíveis, problemas no desempenho escolar ou profissional, além da exposição a outras drogas (OMS, 2018). Embora esses problemas não sejam exclusivos dos adolescentes, eles são mais vulneráveis devido à falta de experiência com os efeitos do álcool (Chung et al., 2018).

No campo da epidemiologia do álcool, é fundamental conceituar e definir o "consumo exacerbado", já que essa caracterização permite avaliar melhor as consequências do uso de EtOH (Axley et al., 2019). Pode-se dizer que a definição de "consumo excessivo de álcool" evoluiu ao longo do tempo. Na década de 1960, cinco doses ou mais de álcool eram consideradas "ingestão pesada", e o consumo excessivo por vários dias era descrito como "*binge*". Foi somente nos anos 1990 que Wechsler e colaboradores (1995) introduziram o termo "binge drinking", para descrever o consumo de uma grande quantidade de álcool em uma única ocasião (Kuntsche, 2017).

O conceito atual foi descrito pelo NIAAA em 2004, que definiu o *binge drinking* como o consumo episódico de álcool que eleva a concentração de EtOH no sangue a 0,08% ou 0,08 g/dL. Esse nível corresponde aproximadamente a quatro ou mais doses de álcool para mulheres e cinco ou mais para homens em um período de duas horas, seguido por um intervalo de abstinência (NIAAA, 2004; Kuntsche et al., 2017; Chung et al., 2018).

Ao aplicar a definição de *binge drinking* no campo de pesquisa, é importante considerar algumas variáveis, como: diferentes nomenclaturas utilizadas para descrever o mesmo padrão de consumo; a estimativa do número de doses por sexo, que muitas vezes desconsidera a concentração de EtOH presente na bebida; e as variações na quantidade de álcool puro em uma unidade de bebida alcoólica, o que é comum ao comparar o consumo entre diferentes países (Kuntsche et al., 2017).

Quando aplicamos a definição de *binge drinking* à faixa etária da adolescência (Blakemore, 2014), observamos que o consumo de álcool entre jovens é proporcionalmente maior do que entre adultos. Isso ocorre porque adolescentes atingem a concentração de 0,08 g/dL ou mais com uma quantidade menor de bebidas alcoólicas, devido ao menor tamanho corporal e às defesas metabólicas menos desenvolvidas (Donovan, 2009).

O padrão de consumo *binge* é particularmente prevalente entre jovens e adolescentes (NIAAA, 2007). De acordo com a Pesquisa Nacional sobre Uso de Drogas e Saúde (2021), 21,5% dos indivíduos nos Estados Unidos com 12 anos ou mais relataram ter consumido álcool em excesso no mês anterior à pesquisa, sendo que a população feminina apresentou uma média de três episódios de *binge drinking* por mês.

De acordo com dados do Centro de Informações sobre Saúde e Álcool (CISA, 2019) e da Organização Mundial da Saúde (OMS, 2017), observa-se uma redução global no consumo excessivo e episódico de álcool entre jovens de 15 a 19 anos, passando de 17,1% em 2000 para 13,6% em 2016. No entanto, essa tendência global de queda não é uniforme. No Brasil, a proporção de jovens dessa faixa etária que relataram consumo abusivo aumentou de 12,7% em 2010 para 18,2% em 2016, contrastando com o padrão global. Além disso, regiões como o Sul e Leste Asiático e o Oeste do Pacífico apresentam estabilidade ou aumento no consumo, reforçando a necessidade de políticas regionais adaptadas ao contexto local (Quadro 1).

	Região OMS	2000	2005	2010	2016
15 – 19 anos	África	17,3	15,7	14,3	12,7
	Américas	25,5	23,4	21,4	18,5
	Leste do Mediterrâneo	0,4	0,3	0,2	0,2
	Europa	35,1	33,5	29,0	24,1
	Sul e Leste Asiático	10,2	9,6	10,4	10,2
	Oeste do Pacífico	18,1	16,2	20,3	18,8
20 - 24 anos	**Mundo**	**17,1**	**15,6**	**15,6**	**13,6**
	África	26,9	24,8	22,9	20,8
	Américas	36,3	33,4	31,2	28,8
	Leste do Mediterrâneo	0,9	0,8	0,7	0,5
	Europa	46,0	44,2	40,0	33,9
	Sul e Leste Asiático	17,4	16,6	17,8	17,6
	Oeste do Pacífico	27,2	24,7	29,9	28,2
	Mundo	25,8	23,7	24,2	21,8

Quadro 1. Dados referentes ao consumo pesado episódico a nível global.
Fonte: OMS, 2017.

No Brasil, dados recentes também demonstram destaque no consumo de bebidas alcoólicas entre as mulheres. De acordo com o relatório Instituto Nacional de Ciência e Tecnologia para Políticas Públicas de Álcool e outras Drogas (INPAD) 2014, houve um crescimento significativo no uso de álcool entre a população feminina - em 2006 representava 27% e no relatório de 2012, correspondia a 38%. Para além, outro dado que merece atenção corresponde ao crescimento de indivíduos que experimentaram bebidas alcoólicas prematuramente, destaque novamente para as mulheres, na qual a

proporção das que experimentaram bebidas alcoólicas antes dos 15 anos passou de 8% em 2006 para 17% em 2012 (INPAD, 2014). Um estudo mais atualizado, da Pesquisa Nacional de Saúde do Escolar (PeNSE) (2019), apresenta diferenças significativas quanto ao ato de experimentar álcool: meninas demonstram percentual de 66,9%, enquanto em meninos, esse percentual foi de 59,6% (IBGE, 2019).

É importante destacar que o organismo feminino possui menores quantidades de enzimas responsáveis pelo metabolismo do EtOH, o que faz com que a droga permaneça por mais tempo na circulação sanguínea até ser completamente metabolizada (NIAAA, 2007; CISA, 2017). Além disso, as mulheres têm, proporcionalmente, menos água no organismo em comparação aos homens, o que eleva a concentração de álcool no sangue e intensifica os efeitos do EtOH. Dessa forma, ao considerar aspectos fisiológicos, pode-se afirmar que o sexo feminino tende a ser mais sensível aos efeitos do álcool em relação ao sexo masculino (NIAAA, 2007).

Fatores que interferem no processo metabólico podem aumentar o risco de certos indivíduos desenvolverem problemas relacionados ao álcool. Independentemente da quantidade ingerida, o organismo pode metabolizar o álcool de forma parcial em um período que varia de acordo com características individuais, como massa corporal e tamanho do fígado (Edenberg, 2007; CISA, 2017).

1.2 Fígado e Metabolismo do álcool

O fígado é o maior órgão interno do corpo humano, situado na região superior direita da cavidade abdominal, abaixo do diafragma. Dividido em lobos, é um órgão composto por unidades funcionais chamadas lóbulos hepáticos, que são constituídos de hepatócitos – células especializadas que desempenham diversas funções metabólicas (Sumadewi, 2023).

Microscopicamente, o tecido hepático saudável apresenta uma organização característica em lóbulos hepáticos. Essas estruturas têm formato hexagonal e são formadas por hepatócitos – células poliédricas com citoplasma acidófilo devido à alta concentração de mitocôndrias e glicogênio. Os hepatócitos estão dispostos radialmente ao redor de uma vênula central, intercalados por sinusoides hepáticos, capilares especializados que facilitam a troca de substâncias entre o sangue e as células hepáticas. Ao longo desses sinusoides, estão localizadas as células de Kupffer e as células estreladas hepáticas. Na periferia dos lóbulos, encontram-se os espaços porta, que proporcionam irrigação sanguínea e linfática do tecido (Sumadewi, 2023).

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Este tecido é vascularizado pelo sistema porta-hepático e pela artéria hepática, que recebe fluxo sanguíneo do trato gastrointestinal e sangue oxigenado diretamente da circulação arterial (Sumadewi, 2023). Esse órgão exerce um papel central na homeostase do organismo, atuando no metabolismo de carboidratos, lipídios e proteínas, na síntese de fatores de coagulação, na produção de bile para a digestão de gorduras e na desintoxicação de substâncias tóxicas (Tso and Mcgill, 2003).

No metabolismo do álcool, os hepatócitos convertem o etanol em acetaldeído por meio da enzima álcool-desidrogenase (ADH) e, posteriormente, em ácido acético, por ação da aldeído-desidrogenase (ALDH). Durante esse processo, ocorre a geração de espécies reativas de oxigênio (ROS) e a depleção de moléculas antioxidantes, o que torna o fígado vulnerável a danos oxidativos, inflamação e lesões estruturais (Cederbaum et al., 2012).

EtOH é completamente absorvido pelo trato digestivo (Bujanda et al., 2000). Esse processo ocorre quando a molécula, que é pequena e anfifílica, atravessa a mucosa digestiva sem necessidade de prévia digestão (Cederbaum et al., 2012) A velocidade de absorção depende de variáveis como a concentração de EtOH, o pH do meio e o tipo de bebida consumida (Paton et al., 2005). Quando a absorção ocorre no intestino, ela é independente da concentração de álcool e da presença de alimentos. No estômago, porém, a absorção depende da repleção ou vacuidade do órgão (Cederbaum et al., 2012)

Apenas de 2% a 10% do EtOH ingerido é eliminado pelos rins e pulmões, enquanto a maior parte é metabolizada pelo fígado, predominantemente nos hepatócitos, embora pequenas quantidades possam ser oxidadas no estômago (Mincis et al., 2011). Vale destacar que o processo de metabolização do álcool é influenciado por fatores como variações nas enzimas que o metabolizam, fatores ambientais, a quantidade de álcool consumida e o estado nutricional do indivíduo (CISA, 2015; NIAAA, 2017). A metabolização do EtOH envolve várias enzimas e cofatores, incluindo ADH, ALDH, o sistema microssomal de oxidação do EtOH (MEOS), que induz o citocromo P4502E1, além dos cofatores NAD e NADP+O2 (Hyun et al., 2021).

1.3 Oxidação do EtOH

O EtOH é desintoxicado e eliminado principalmente no tecido hepático, através de uma série de alterações metabólicas de cunho oxidativo (Cederbaum et al., 2012). Esse processo acontece por meio de três vias principais, que se desenvolvem em três fases distintas (Mincis et al., 2011; Figura 1).

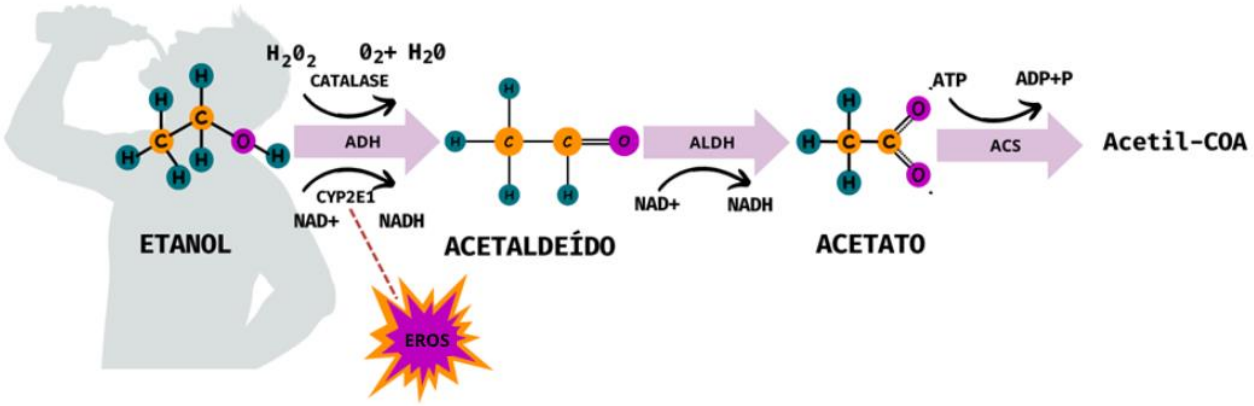

Figura 1. Ilustração da via metabólica da oxidação do EtOH. ADH: Álcool Desidrogenase; CYP2E1: Citocromo P450 2E1; ALDH: Aldeído Desidrogenase; ACS: Acetil-CoA Sintetase; ERO: Espécies Reativas de Oxigênio

Fonte: Autor *et al.*, 2024

A primeira etapa do metabolismo do EtOH começa com a ação da enzima citosólica álcool desidrogenase (ADH), que converte o EtOH em acetaldeído, como mostrado na equação a seguir:

EtOH + NAD+ → acetaldeído + NADH + H+

Aqui, observa-se a formação de acetaldeído e de NADH, produto da oxidação de um mol de EtOH para cada mol de NAD^+ consumido (Cederbaum, 2012; Figura 1). Essa via é predominante entre os bebedores sociais, pois a disponibilidade de NAD^+ no organismo é limitada (Mincis et al., 2011). Além de gerar energia (16 ATP/mol de EtOH), essa via também regula o processo oxidativo.

No entanto, em situações de consumo excessivo ou crônico de álcool, a capacidade da ADH pode se esgotar. Nessas condições, o fígado utiliza de outras duas vias metabólicas: o sistema oxidativo de EtOH microssomal (MEOS) e a via de catalase (Mincis et al., 2011; Zakhari, 2016; Figura 1).

A segunda via, o MEOS, localiza-se no retículo endoplasmático dos hepatócitos e é ativada principalmente em consumidores crônicos de álcool (Cederbaum et al., 2012). Embora essa via seja suplementar, ela é capaz de metabolizar até 20% do EtOH consumido em excesso, utilizando o cofator Nicotina-Adenina-Dinucletídio-Fosfoto Hidrogenado (NADPH) e o complexo enzimático citocromo P-450 (CYP2E1), NADPH redutase e fosfolipídeos (Lieber, 1995). A equação que resume essa via é:

EtOH+ NADPH + H+ + O2 → acetaldeído + NADP+ + 2H2 O

Diferente da via ADH, o sistema MEOS consome energia, sem gerar NADH, o que representa uma diferença importante para o metabolismo hepático, especialmente em bebedores crônicos (Cederbaum, 2012). Além disso, sua via metabólica atua em outros substratos no nosso organismo, como em esteroides, ácidos graxos e xenobióticos (USP, 2020).

Por fim, a terceira via, mediada pela catalase, tem uma participação reduzida na oxidação do EtOH. Ela depende do peróxido de hidrogênio (H_2O_2) para oxidar o EtOH, gerando acetaldeído e água:

EtOH + H2O2 → acetaldeído + 2H2O

Apesar de sua contribuição pequena (menos de 2% do EtOH metabolizado), a via da catalase pode se tornar relevante em condições de estresse oxidativo (Cederbaum, 2012).

Independentemente da via utilizada, o produto da primeira etapa do metabolismo do EtOH é sempre o acetaldeído, uma substância altamente reativa e tóxica. Na segunda etapa, o acetaldeído é oxidado pela enzima aldeído desidrogenase, gerando acetato, um metabólito tóxico que pode ser utilizada no organismo como fonte de energia (Figura 1):

Acetaldeído + NAD+ → acetato + NADH + H+

A reação de oxidação do álcool até a formação do acetato gera 2 moles de NADH + H+/mol de EtOH, o que propicia um desequilíbrio no quociente NADH/NAD+. O aumento de NADH levará à formação de acetaldeído e proliferação microssomal através da via MEOS. Por outro lado, como NAD é necessário em outras reações metabólicas e sua biodisponibilidade é limitada, o desbalanço na relação NADH/NAD+, acaba promovendo a inibição da síntese de ácidos graxos e proteínas, aumento a peroxidação lipídica, formação de radicais livres, entre outras que integram o conjunto de mecanismos capazes de causar dano ao fígado (Matos, 2003; Mincis et al., 2011; Lanza et al., 2021).

1.4 Hepatotoxicidade do EtOH

A hepatotoxicidade do álcool está intimamente relacionada ao seu metabolismo, resultando em produtos que incluem espécies reativas de oxigênio e nitrogênio, bem como à depleção de cofatores, como o NAD^+, e alterações na homeostase do organismo (Ceni et al., 2014; Hyun et al., 2018).

Os subprodutos das vias oxidativas causam uma perturbação metabólica específica e tóxica, sendo o acetaldeído o principal produto gerado (Albano, 2006). Este metabólito é crucial na hepatotoxicidade do EtOH, pois pode danificar o tecido hepático, desencadeando inflamação, remodelação da matriz extracelular e fibrogênese (Lieber, 2005; Cederbaum, 2012).

O acetaldeído é responsável por efeitos sistêmicos observados após o consumo excessivo de álcool, como náuseas, tontura e dores de cabeça, frequentemente relatados após o consumo excessivo de álcool (Eriksson et al., 2001). Em nível molecular, este metabólito tóxico pode se ligar a proteínas, lipídios e ao DNA, provocando danos estruturais. Ele também interfere no metabolismo de ácidos graxos e na respiração celular, favorecendo a produção de espécies reativas de oxigênio, que agravam o estresse oxidativo no fígado (Anni et al., 2003; Cederbaum et al., 2012; Hyun et al., 2018; Figura 2).

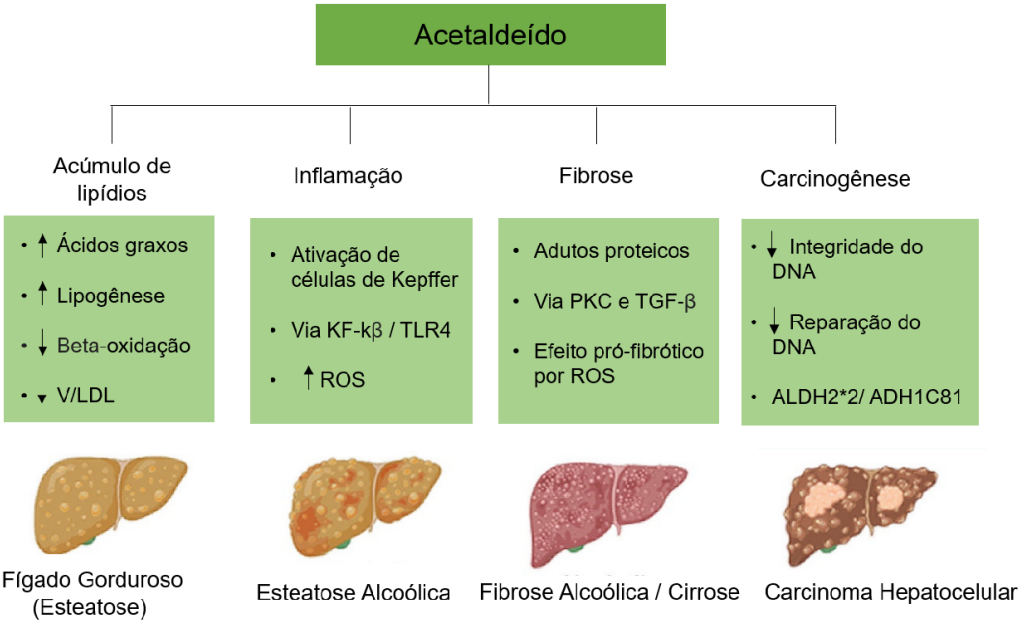

Figura 2. Efeitos nocivos do acetaldeído no tecido hepático. KF-κβ: Fator de Transcrição Nuclear Kappa B; TLR4: Receptor Toll-like 4; ROS: Espécies Reativas de Oxigênio; PKC: Proteína Quinase

C; TGF-β: Fator de Crescimento Transformador Beta; ALDH2: Aldeído Desidrogenase 2; ADH1C81: Álcool Desidrogenase 1C (Isoforma 81)
Fonte: Adaptado de Hyun *et al.* 2021.

Outro fator que contribui para a hepatotoxicidade associada ao álcool é a desestabilização do quociente $NADH/NAD^+$. Esse desequilíbrio pode desencadear uma série de alterações fisiológicas, incluindo mudanças na expressão gênica no núcleo, comprometimento da função mitocondrial e hipóxia tecidual, especialmente em hepatócitos perivenosos. Esses hepatócitos são particularmente suscetíveis a danos precoces decorrentes do uso de álcool, o que torna essa região do fígado mais vulnerável à lesão (Xu et al., 1994; Hyun et al., 2018). Entretando, a literatura não demonstra qual o impacto do consumo excessivo episódico na região perivenosas.

A conversão oxidativa de EtOH em acetato, além de ser responsável pela formação de dióxido de carbono (CO_2), ácidos graxos (FAs) e água (H_2O) nos tecidos periféricos, leva à produção de acetil coenzima A. Esse produto metabólico promove a acetilação de histonas, o que desencadeia processos inflamatórios no fígado (Cederbaum et al., 2018). A acetilação de histonas, particularmente em promotores de genes específicos, é crucial na regulação de citocinas pró-inflamatórias, como o fator de necrose tumoral alfa (TNF-α), interleucina (IL)-1β e IL-8, comumente observadas em pacientes com doença hepática alcoólica (Seth et al., 2001; Gao et al., 2011).

Além disso, os subprodutos do metabolismo do EtOH, como o acetaldeído, podem gerar interrupções nas vias de sinalização celular e comprometer a função dos canais iônicos, agravando o estresse oxidativo. Esse estresse, por sua vez, ativa a resposta imune adaptativa, em grande parte desencadeada pela formação de adutos proteicos de acetaldeído, como o malondialdeído e hidroxinonenal. Esses adutos afetam diretamente a função celular e contribuem para a progressão da lesão hepática (Ceni et al., 2014; Lherena et al., 2016).

O acetaldeído, principal metabólito do EtOH, também é responsável pela ativação do fator de crescimento transformador beta (TGF-β) em células estreladas hepáticas. Essas células, ao serem estimuladas, tornam-se as principais produtoras de colágeno e fibronectina, favorecendo o desenvolvimento de processos inflamatórios e fibrogênicos no fígado (Osna et al., 2017; Parola & Pinzane, 2019).

O consumo de álcool exerce um impacto significativo no metabolismo lipídico hepático, sendo um fator crucial no desenvolvimento da DHA. O desequilíbrio na razão NADH/NAD+ interfere nas vias de oxidação lipídica, resultando no acúmulo de triglicerídeos nos hepatócitos, característica típica da esteatose hepática alcoólica (Cederbaum et al., 2012). Esse quadro está intimamente relacionado a alterações na expressão de genes envolvidos no metabolismo lipídico. Um dos principais genes afetados é o da proteína de ligação ao elemento regulador de esterol-1 (SREBP-1), que regula a síntese de ácidos graxos e triglicerídeos (Purohit et al., 2008). O consumo de álcool aumenta a atividade do SREBP-1, um efeito mediado pela inibição da proteína quinase ativada por AMP (AMPK) e da sirtuína-1, que, em condições normais, atuam para regular negativamente essa proteína. Além disso, o álcool pode diminuir a atividade do receptor-α ativado por proliferador de peroxissoma (PPAR-α), um regulador crucial da oxidação de ácidos graxos, contribuindo ainda mais para o acúmulo de lipídios no fígado (Purohit et al., 2008).

O desequilíbrio no metabolismo lipídico pode progredir para alterações inflamatórias e fibrosas no fígado, características de formas mais graves da DHA, como a hepatite alcoólica e a cirrose (Tsukamoto et al., 2017). A interação entre estresse oxidativo, inflamação e desregulação da homeostase lipídica sublinha o impacto do álcool nos organismos vivos.

Em termos macroscópicos, o consumo prolongado de álcool pode resultar em diversas alterações no fígado, como acúmulo de gordura (esteatose), inflamação, perda de função, necrose e fibrose, dependendo da gravidade da lesão (Gao et al., 2011). Diferentes mecanismos descritos estão interligados e são fundamentais para a compreensão de como o consumo de álcool perpetua e agrava as lesões hepáticas, conforme evidenciado no quadro 2.

Mecanismos associados à lesão hepática
Desequilíbrio do quociente NADH/NAD
Estresse oxidative
Formação de acetaldeído
Proliferação microssomal no hepatócito
Alterações de membrana
Retenção de proteínas e água no hepatócito
Estado hipermetabólico
Aumento da deposição de gordura
Alterações imunológicas
Formação de fibrose
Efeitos de citocinas
Apoptose hepática e de células mononucleares no sangue
Desnutrição

Quadro 2. Mecanismos associados ao dano hepático resultante do consumo de EtOH.
Fonte: Adaptado de Mincis (2011)

Um estudo conduzido por Bertolini e colaboradores (2014) indicou que as mulheres apresentam maior propensão a episódios de bebedeira em relação aos homens. Além disso, começaram a exibir padrões de consumo semelhantes aos de seus pares masculinos, especialmente no que se refere ao consumo episódico pesado (Nixon et al., 2023).

É fundamental salientar que o sexo feminino é mais vulnerável aos danos hepáticos induzidos pelo álcool, como demonstrado no Quadro 3, em função de fatores fisiológicos específicos. Entre esses fatores estão o menor volume de água e massa corporal, que resulta em uma concentração maior de álcool no sangue; a menor atividade da enzima (ADH) gástrica, o que reduz a metabolização inicial do álcool no estômago; a influência dos estrógenos, que facilita a endotoxemia; e a desaceleração do

esvaziamento gástrico durante a fase lútea do ciclo menstrual, o que favorece uma maior absorção de EtOH antes que ocorra sua metabolização (Bradley et al., 1998; Fulham & Mandrekar, 2016; Ohasi et al., 2018; Sharma & Arora, 2020).

Fatores que influenciam dano hepático
Quantidade álcool ingerida
Duração (tempo) de ingestão
Continuidade
Sexo feminine
Substância hepatóxica na bebida alcoólica
Outras patológicas, bem como: obesidade e deposição de ferro
Hepatites virais
Fator genético (predisposição)
Desnutrição

Quadro 3. Fatores que influenciam o dano hepático resultante do consumo de EtOH.
Fonte: Adapto de Mincis (2011).

Embora a literatura seja clara sobre os danos hepáticos resultantes do consumo excessivo e crônico de EtOH (Yang et al., 2014; Li et al., 2017; Fernandes et al., 2018), os efeitos de episódios repetidos de consumo em padrões binge durante a adolescência e sua repercussão na função hepática na fase adulta ainda não estão totalmente descritos. Nosso grupo demonstrou que ciclos repetidos de consumo de EtOH na adolescência podem induzir lesões hepáticas em ratas fêmeas (Fernandes et al., 2018) mas os impactos do consumo em padrão *binge* ao longo da vida ainda carecem de investigação. Assim, o presente estudo visa investigar as alterações hepáticas associadas ao consumo intermitente e episódico de EtOH, desde a adolescência até a fase adulta, e avaliar se essas alterações persistem após um longo período de abstinência.

Objetivos

2.1 Objetivo geral:

Investigar o consumo intermitente e episódico de EtOH em ratas da adolescência à fase adulta e suas repercussões bioquímicas e morfológicas no tecido hepático, a curto e longo prazo.

2.2 Objetivos específicos:

- Desenvolver análise bibliométrica acerca do consumo de EtOH no padrão *binge drinking* e possíveis alterações hepáticas.
- Mapear o conhecimento do efeito dos diferentes protocolos de consumo intermitente e episódico de álcool sobre a função hepática.
- Investigar as possíveis alterações hepáticas a curto e a longo prazo relacionadas ao consumo de EtOH em padrão *binge* em marcadores bioquímicos hepáticos periféricos em modelo experimental.
- Determinar possíveis impactos no metabolismo lipídico em modelo murino.
- Investigar o balanço oxidativo do tecido hepático de ratas intoxicadas com EtOH, através da quantificação indireta de peroxidação lipídica, e as repercussões a longo prazo.
- Determinar a atividade das enzimas antioxidantes frente ao desequilíbrio redox induzido pelo consumo de álcool a curto e a longo prazo em padrão *binge* em animais experimentais.
- Descrever a histopatologia do tecido hepático de ratas adultas intoxicadas com EtOH, da adolescência à fase adulta.

2. **Resultados**

3.1 Estudo bibliométrico

O artigo a seguir foi desenvolvido como primeiro capítulo da dissertação intitulado “What is known about binge drinking and liver function? A bibliometric approach of the 100 most cited articles, past and future trends”. O presente trabalho teve como objetivo o desenvolvimento de estudo bibliométrico sobre o consumo de EtOH em padrão *binge* e suas repercussões hepáticas.

Este documento foi submetido ao periódico “Pharmacological Research” que possui Fator de Impacto de 9.1 e com Score de Citação de 18.7. O documento comprobatório está contido no Anexo I.

What is known about binge drinking and liver function? A bibliometric approach of the 100 most cited articles, past and future trends.

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Abstract

Alcohol consumption has been culturally accepted for centuries due to its psychotropic effects and legal status. Alcohol intake, mostly in a binge-like manner, has demonstrated several risks for the development of addiction, immunological disturbances, neurophysiological insults, and particularly hepatotoxicity. We conducted a bibliometric analysis of the 100 most cited articles related to binge drinking and hepatotoxicity. We retrieved articles included in the criteria from the Web of Science Core Collection (WoS-CC) database, following the bibliometric rules, such as number of annual publications and citations, authors' productivity, keyword analysis, journals of publication, geographic distribution, and funding agencies. Critical analysis focused on study types and binge-type protocol. Results indicated Gao B as the most prolific author, with 18 publications and 2,559 citations. The journal with the most papers published was Hepatology, and the more frequent keywords were “liver steatosis” and “alcohol”. Geographic aspects, the United States was the prominent country of publications, with 68 articles and 7,438 citations. The analysis of funding agencies confirmed that the National Institutes of Health (NIH) stood out as the largest scientific funder. Content mapping identified five categories of binge-like ethanol protocols, with “binge ethanol” and “chronic plus binge ethanol feeding” as the most common models for inducing hepatotoxicity. Our data highlighted the scenario of the most cited scientific papers related to binge drinking and liver dysfunction, evidencing that the extent and mechanisms of liver damage vary depending on the binge drinking pattern, with inflammatory processes, oxidative stress, and lipid metabolism dysregulation the main source of hepatotoxicity.

Keywords: Binge drinking, liver changes, hepatoxicity, oxidative stress, alcohol consumption.

1 Introduction

Alcohol is a widely consumed psycho-depressant drug due to its licit social condition in most countries, easily accessible and the consumption encouraged and consolidated by centuries-old cultural practices [1, 2, 3]. Unbridled alcohol consumption emerges as the main risk factor for physical disability and premature death, accounting for 5.3% of annual deaths worldwide, beyond significant physical injuries and the development of more than 200 diseases [2]. Therefore, the harmful use of alcohol is considered a serious but preventable public health problem worldwide [4, 5].

The threshold between social consumption and chemical dependence on alcohol is not well defined, depending not only on the volume and concentration ingested but also on the frequency, period of life, and circumstances in which the drug is used. Some of these variables establish the definition of ethanol consumption patterns [6]. Albeit there are no safe levels of alcohol consumption, even in minimal doses, the substance can produce significant physiological changes [2]. Moderate intake has been defined as solely one dose for women and 2 drinks for men [7]. Contradictory, the National Institute of Alcohol Abuse and Alcoholism [6] has defined the pattern of binge drinking for those drinkers for which the blood alcohol concentration (BAC) reaches at least 0.08g/dL, equivalent to a minimum of 4 drinks for women and 5 doses for men, for a couple of hours [6]. In the occurrence of a binge-like episode reaching the BAC above 0.16g/dL, a more detrimental binge-type category occurs, called high-intensity drinking [6]. An additional profile of heavy alcohol consumption has also been seen with lower frequency, related to 15 or above drinks for men and 8 or above drinks for women [6]. Therefore, understanding patterns of alcohol consumption is fundamental to attributing the burden of consumption to its causal factors, so that different patterns of consumption can produce different physiological damage [8, 9].

Among the consumption classifications, binge drinking demonstrates an additive risk of disease and injury when compared to other patterns due to the intermittent and socially tolerable standard [8]. In addition to economic and social losses and a greater probability of developing psychological disorders [10, 11]. These findings are justified by the physiological changes that occur in intoxication due to all spectrum of the binge drinking profile, such as immune disorders and changes in cardiovascular and liver mechanisms, as well as neurophysiological changes that generate cognitive impairment, reduced judgment, motor impairment, and attention failures which can present a long-

lasting profile, as widely reported in experimental and clinical studies [12, 13, 14, 15, 8, 16, 17, 18, 19, 20, 21].

Ethanol is detoxified mainly in liver tissue through oxidative metabolic changes. The canonic alcohol oxidation pathway, commonly activated in social drinkers, occurs by the oxidative process through the alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) enzymes. Such enzymatic oxidation requires the coenzyme nicotinamide adenine dinucleotide (NAD), which is subject to saturation (limited pathway). In the binge drinking profile, oxidative complementary pathways are activated, such as the microsomal via, composed mainly of cytochrome P450 2E1 (CYP2E1) considered a toxic system, which through the active processes through the expenditure of adenosine triphosphate (ATP) metabolizes alcohol with consequent production of reactive oxygen species. In turn, the catalase enzymatic pathway present in peroxisomes is responsible for the oxidation of less than 2% of the alcohol ingested. This pathway requires the presence of hydrogen peroxide, which has low generation rates produced under physiological cellular conditions [22, 23, 24] (Figure 1).

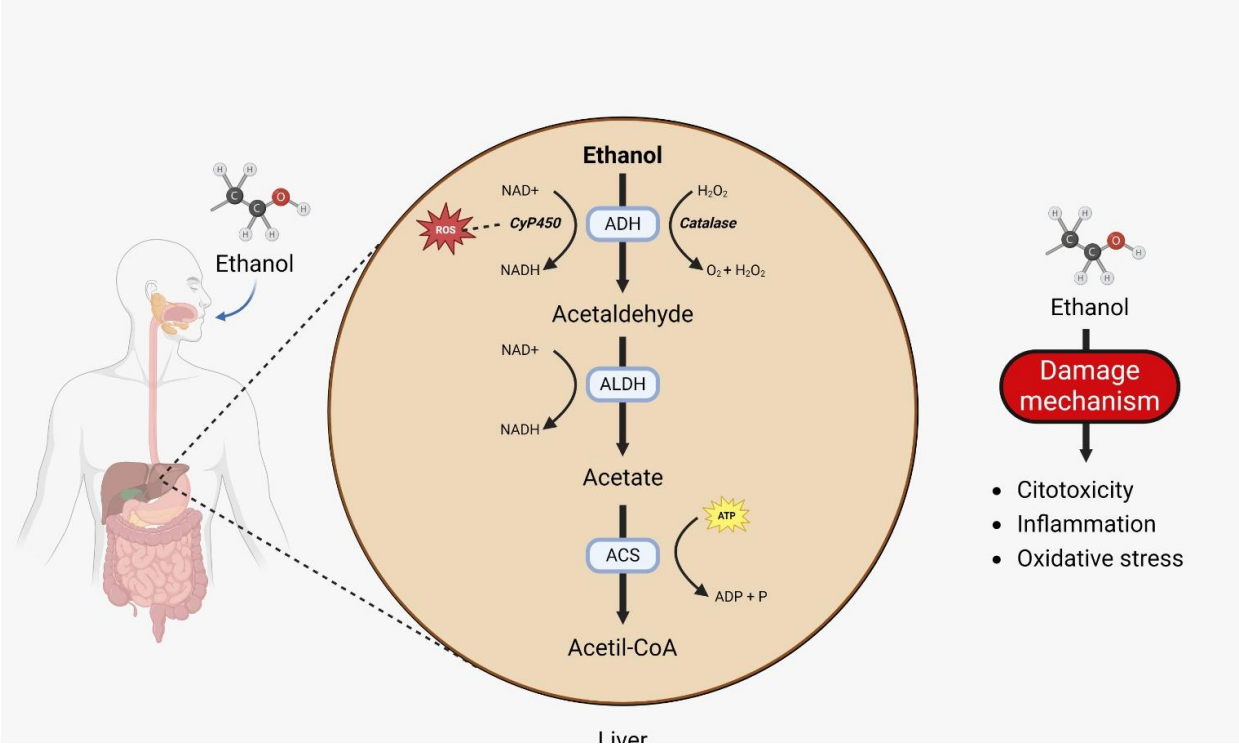

Figure 1. Ethanol metabolism in the liver. The image shows a simplified diagram of ethanol metabolism in the liver and the main mechanisms by which binge drinking can damage this organ. ADH: alcohol dehydrogenase; ADP: adenosine diphosphate; ALDH: aldehyde dehydrogenase; ATP: adenosine triphosphate; CYP2E1: cytochrome P450 E1; NAD: adenine dinucleotide.

Although the products of alcohol metabolization are harmful to different organs and tissues, the liver is especially susceptible to damage, as it is considered a “frontline” organ in the metabolization and neutralization of alcohol toxic metabolites [22, 25, 26, 23, 27, 28, 29]. In this way, excessive alcohol intake can produce physiological changes through oxidative imbalance, as well as cytotoxicity and inflammatory processes [30, 31, 32, 26, 29, 33, 34, 35].

Binge drinking profiles have increased considerably in recent decades, resulting in pronounced hepatic implications. Although studies on this mode of ethanol consumption and metabolism have been significantly advanced, there is still no bibliometric analysis that comprehensively provides the relevant findings of the most reported articles on this subject. Thus, the aim of this study is to conduct a bibliometric analysis to identify, evaluate, and map the 100 most cited articles investigating ethanol consumption in binge drinking protocols and its liver alterations. The investigation of the most mentioned documents allows us to understand new trends in the scientific field, as they represent the significant impact of the articles in the area. This approach will allow a more detailed and integrated understanding of scientific advances in this field, as well as the identification of gaps and prospects for further research on the alcohol and liver health.

Material and methods

This bibliometric analysis was conducted based on the recently published guideline titled Preliminary Guideline for Reporting Bibliometric Reviews of the Biomedical Literature (BIBLIO): A Minimum Requirements [36], with the aim of providing greater transparency in the presented data through more detailed instructions on the mapping conducted.

Search

The data for this research was retrieved in February 2024 from the Web of Science Core Collection (WoS-CC) database. We conducted searches using terms related to binge drinking and liver changes (Table 1), identifying a total of 972 articles.

Table 1. Search strategy.

Database	Search strategy
WoS- Core Collection	TS=(Liver OR Livers OR Hepatocytes OR Hepatocyte OR "Hepatic Cells" OR "Cell, Hepatic" OR "Cells, Hepatic" OR "Hepatic Cell" OR "Liver Failure" OR "Hepatic Failure" OR "Hepatic INJURY" OR "End Stage Liver Disease" OR "Chronic Liver Failure" OR "Chronic Liver Failures" OR "Failure, Chronic Liver" OR "Failures, Chronic Liver" OR "Liver Failures, Chronic" OR "Liver Failure, Chronic" OR "Liver Failure, Acute" OR "Failure, Acute Liver" OR "Fulminant Hepatic Failure" OR "Fulminant Hepatic Failures" OR "Failure, Acute Liver" OR "Fulminant Hepatic Failure" OR "Fulminating Hepatic Failure" OR "Fulminating Hepatic Failures" OR "Hepatic Failure, Fulminating" OR "Fulminating Liver Failure" OR "Fulminating Liver Failures" OR "Liver Failure, Fulminating" OR "Acute Liver Failure" OR "Liver Failure, Fulminant" OR "Fulminant Liver Failure" OR "Fulminant Liver Failures" OR "Hepatic Failure, Acute" OR "Acute Hepatic Failure" OR "Failure, Acute Hepatic" OR "Hepatic Failure, Fulminant" OR "Hepatic Insufficiency" OR "Insufficiency, Hepatic" OR "Liver Insufficiency" OR "Insufficiency, Liver" OR "Liver Cirrhosis" OR "Hepatic Cirrhosis" OR "Cirrhosis, Hepatic" OR "Cirrhosis, Liver" OR "Fibrosis, Liver" OR "Liver Fibrosis" OR "Liver Cirrhosis, Experimental" OR "Cirrhosis, Experimental Liver" OR "Experimental Liver Cirrhoses" OR "Hepatic Cirrhosis, Experimental" OR "Experimental Hepatic Cirrhosis" OR "Liver Cirrhoses, Experimental" OR "Cirrhoses, Experimental Liver" OR "Experimental Liver Cirrhosis" OR "Acute-On-Chronic Liver Failure" OR "Acute On Chronic Liver Failure" OR "Acute-On-Chronic Liver Failures" OR "Failure, Acute-On-Chronic Liver" OR "Failures, Acute-On-Chronic Liver" OR "Liver Failures, Acute-On-Chronic" OR "Liver Failure, Acute-On-Chronic" OR "Liver Failure, Acute On Chronic" OR "Acute-On-Chronic Liver Failure (ACLF)" OR "Acute On Chronic Liver Failure (ACLF)" OR "Acute-On-Chronic Liver Failures (ACLF)" OR "Failure, Acute-On-Chronic Liver (ACLF)" OR "Failures, Acute-On-Chronic Liver (ACLF)" OR "Liver Failure, Acute-On-Chronic (ACLF)" OR "Liver Failures, Acute-On-Chronic (ACLF)" OR "Liver Diseases" OR "Disease, Liver" OR "Diseases, Liver" OR "Liver Disease" OR "Liver Dysfunction" OR "Dysfunction, Liver" OR "Dysfunctions, Liver" OR "Liver Dysfunctions" OR "Alcoholic Liver Diseases" OR "Liver Diseases, Alcoholic" OR "Alcoholic Liver Diseases" OR "Alcoholic Liver Disease" OR "Liver Disease, Alcoholic" OR "Liver Cirrhosis, Alcoholic" OR "Alcoholic Liver Cirrhosis" OR "Hepatic Cirrhosis, Alcoholic" OR "Alcoholic Hepatic Cirrhosis" OR "Alcoholic Cirrhosis" OR "Chemical and Drug Induced Liver Injury, Chronic" OR "Drug-Induced Liver Injury, Chronic" OR "Drug Induced Liver Injury, Chronic" OR "Liver Injury, Drug-Induced, Chronic" OR "Chronic Drug-Induced Liver Injury" OR "Chronic Drug Induced Liver Injury" OR "Hepatitis, Chronic, Drug-Induced" OR "Chemical-Induced Liver Injury, Chronic" OR "Chemical Induced Liver Injury, Chronic" OR "Chemically-Induced Liver Injury, Chronic" OR "Chemically Induced Liver Injury, Chronic" OR "Fatty Liver, Alcoholic" "Alcoholic Fatty Liver" OR "Alcoholic Steatohepatitis" OR "Fatty Liver" OR "Liver, Fatty" OR Steatohepatitis OR Steatohepatitides OR "Steatosis of Liver" OR "Liver Steatosis" OR "Liver Steatoses" OR "Steatoses, Liver" OR "Steatosis, Liver" OR "Massive Hepatic Necrosis" OR "Hepatic Necrosis, Massive" OR "Acute Yellow Atrophy of Liver" OR "Acute Yellow Atrophy" OR "Acute Yellow Atrophies" OR "Yellow Atrophy, Acute" OR "episodes of AH") AND TS=("Binge Drinking" OR Binge OR "Binge Alcohol Consumption" OR "Binge Drinkers" OR "Binge-Like Ethanol" OR "Binge Ethanol" OR "Binge-Like Alcohol" OR "Binge Alcohol" OR "Heavy Episodic Drinking" OR "Episodic Heavy Drinking" OR "Heavy Sessional Drinking" OR "Dangerous Drinking" "Risky Single-occasion Drinking" OR "High-Risk Drinking" OR "Risky Single Occasion Drinking" OR "Excessive Episodic Consumption" OR "Frequent Binge Drinking" OR "Concentrated Drinking Episode" OR "Binge-Drinking" OR "Binge Alcohol Exposure" OR "Binge Ethanol Exposure" OR "Chronic-Binge Ethanol" OR "Chronic Binge Ethanol" OR "Acute Binge Ethanol" OR "Acute-Binge Ethanol" OR "Binge Episode" OR "Binge Alcohol" OR "Binge Administration of Alcohol" OR "Binge Drink" OR "Alcohol Binge Drinking" OR "Intermittent Ethanol Exposure" OR "Binges" OR "Binge/Heavy Drinking" OR "Intermittent Alcohol 'Binge' Drinking" OR "Excessive Consumption Alcohol")

Time Period

No restrictions were applied regarding the publication year during the selection of articles, as a global analysis was chosen to conduct a broader search aimed at selecting the 100 most cited articles on the topic.

Selection of articles

The 100 most cited articles were selected by two blinded researchers independently, who made their decision after reading the title and abstract, followed by an analysis of the full text. In the event of disagreement between the researchers over the inclusion of the document, a third consultant solved the impasse. The inclusion criteria included original and review articles on the subject, with no language restrictions. Exclusion criteria included conference papers, editorials, letters, inaccessible articles, and publications that did not address the central theme (binge drinking and the liver; Figure 2). The excluded documents were recorded in a table containing the title, authors, number of citations, and the reason for the exclusion (Table 1, Supplementary Information).

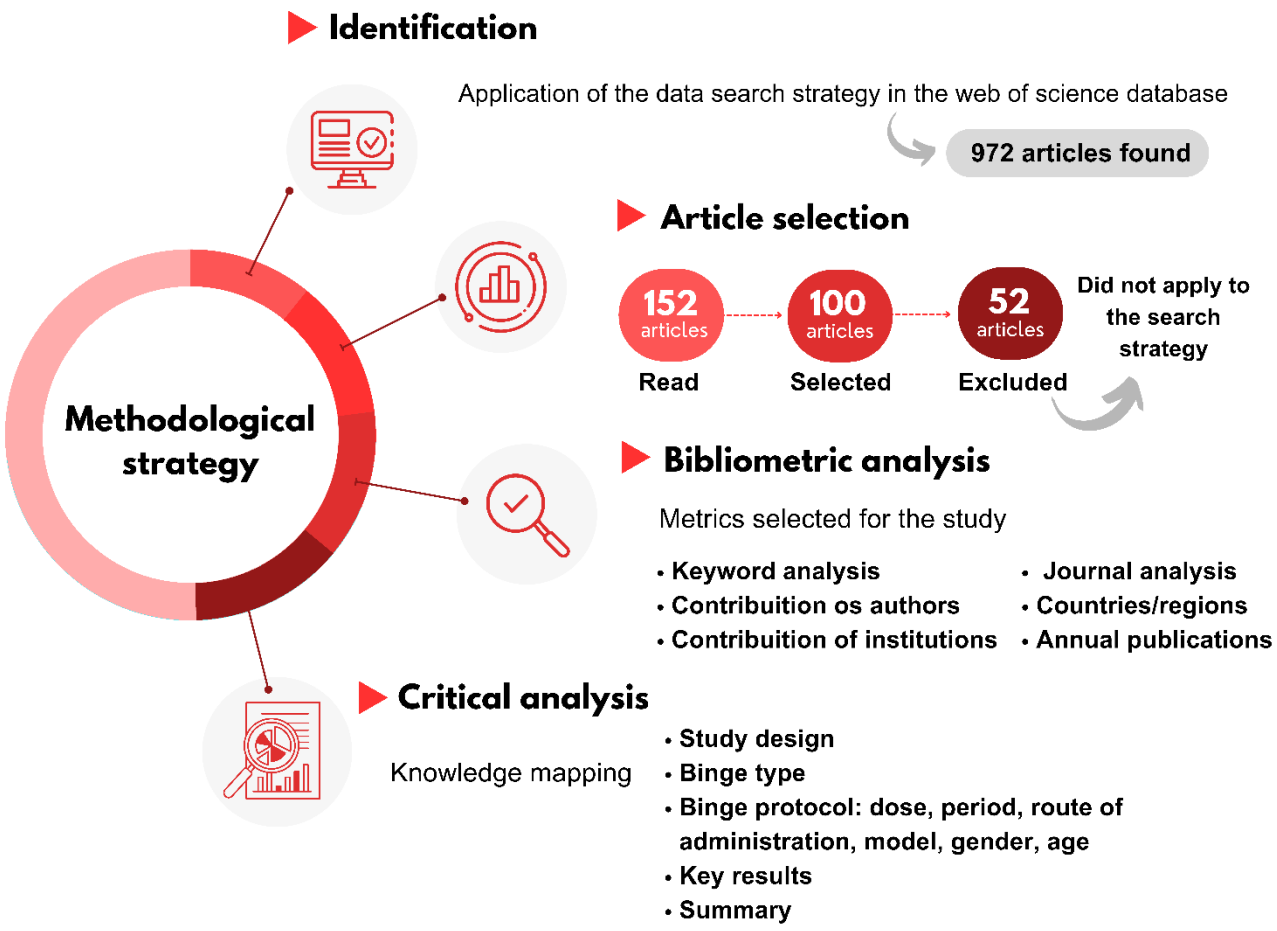

Figure 2. Methodological strategy for data extraction. The image shows the flowchart describing the steps taken to produce this bibliometric survey.

Data extraction - bibliometric parameters

Following selection, the files were extracted from WoS-CC and converted into a document in TXT format, in which the complete record of these articles was retrieved. This file was exported to the Visualization of Similarities Viewer (VOSviewer) software version 1.6.19 - to translate the codes generated into communication and density networks or for descriptive reading in complementary software.

For bibliometric parameters, the keywords and their timeline, interaction networks of authors and co-authors, financial institutions, journals of publication, and the country of the corresponding author were collected. The data obeyed the descending order according to the number of citations in WoS-CC. In case of a tie, the tiebreaker was established by the density of citations.

Data Analysis - bibliometric parameters

To build and visualize the networks, the VOSviewer tool was used, which organizes the information into clusters and their network connections, thus exposing representations in which elements that share similar characteristics are placed closer together. In order to build the authors' keyword network, the TXT document with the articles' records was edited, unifying terms and words with the same meaning, taking care not to bias or modify the results.

To produce the author network, the TXT file was previously adjusted - unifying the identification of authors who previously appeared as similar names. Authors with at least one article were considered as the unit of analysis. The descriptive evaluation of the data, such as journals, financial institutions, and countries of publication, was produced using Microsoft Excel 365 software, and the graphical projection of the countries was produced using MapChart - a map customization tool (mapchart.net/index.html).

Content mapping

The 100 documents chosen were carefully read and information and metrics were summarized in tables to build the information map. The title, authors, year of publication, objective, DOI/URL, number of citations (WoS, Scopus, and Scholar), and citation density (WoS) were recorded. In addition, the type of study and period, binge protocol applied in *in vivo* models - extracting dose, route

of administration, period of exposure to alcohol, model, gender, and age, as well as the record of liver damage induced by the alcohol protocol and the summary of the article was classified.

Through the analysis of the documents, secondary findings were identified and processed using R version 4.3.2 to generate two types of charts: the Sankey diagram and the Circus Plot. For the Sankey diagram, the parameters used were Financing, author, and country. These data were first organized in a Microsoft Excel spreadsheet and then imported into R using the "readxls" package. Once the data were imported, the "networkD3" package was utilized to create the diagram, while "dplyr" was employed for filtering and summarizing the information. For the Circus Plot, the parameters used were Author, Protocol, and Outcome. The organization and processing of this data were carried out with the "circlize" package, which is well-suited for representing relationships between categorical variables, such as the associations between different groups (R Core Team, 2020).

Results

Selected Studies and Bibliometric Analysis

After applying the search strategy, 972 documents related to the topic were obtained, of which 152 articles in descending order based on the number of citations were read, 100 were selected and 52 were excluded. It was considered in the bibliometric analysis citation count and density, author's contribution, institution's contribution, keywords co-occurrence, regions and journals of the publications, and knowledge mapping (Figure 3).

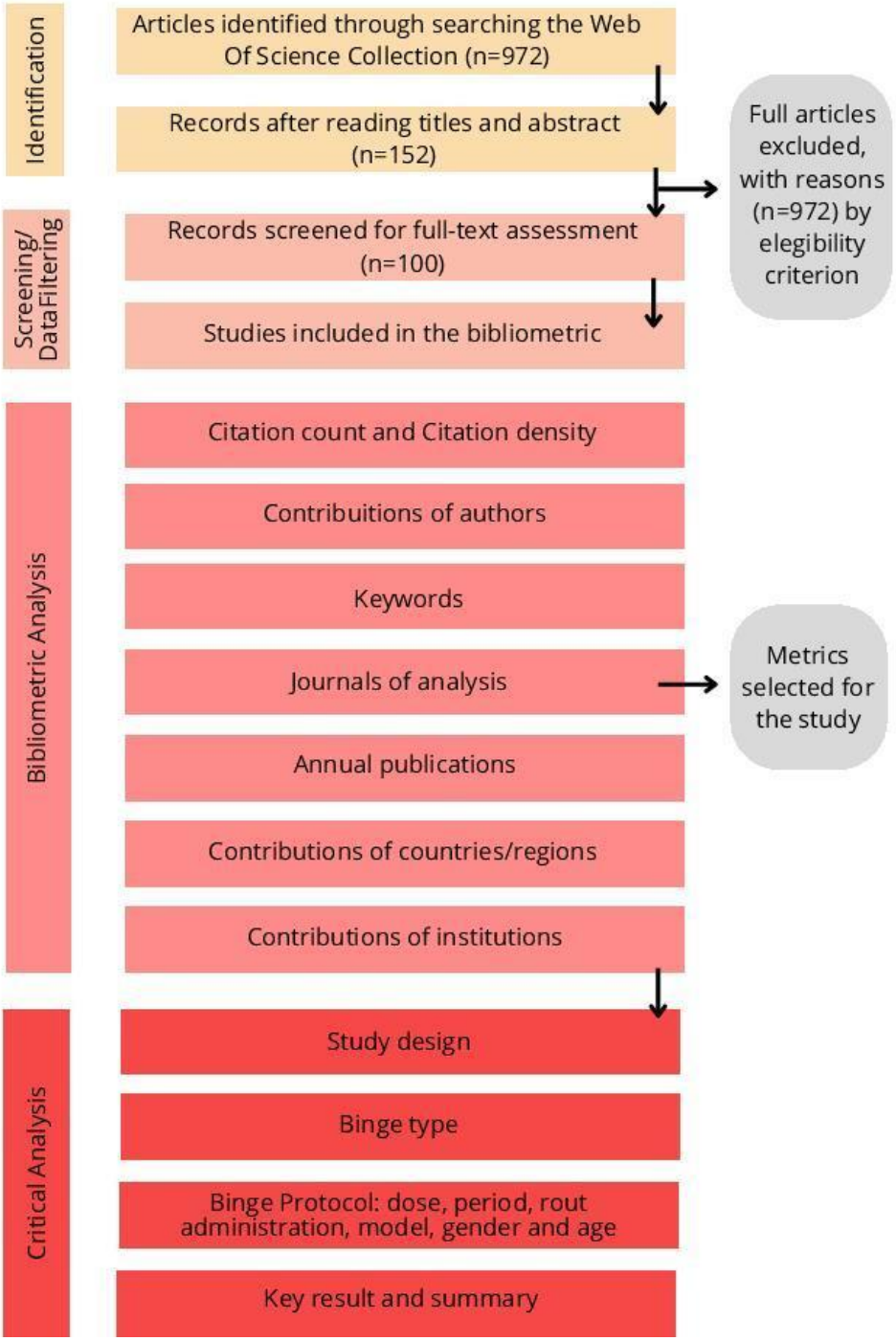

Figure 3: Flowchart of article selection. The figure shows the inclusion criteria and the critical metrics and analyses.

Citations

The 100 selected articles accumulated a total of 10,110 citations. The most cited document, entitled "Mouse model of chronic and binge ethanol feeding (the ref model)", received 679 citations, while the least cited, entitled "Apoptosis of enterocytes and nitration of junctional complex proteins promote alcohol-induced gut leakiness and liver injury", obtained 50 citations. The oldest article, published in 1996, describing a model of binge drinking in mice for immunological evaluation, was cited 130 times, while the most recent, published in 2021, addressing the hepato-physiological aspects of alcohol metabolism, received 82 citations (Table 2).

Table 2. The Top 100 Most cited papers about binge drinking and liver changes considering density citation.

Ranking	Author/Year	Title	Objective	Number of citations			DOI/URL
				WoS-CC (Citation Density)	Scopus	Google Scholar	
1	Bertola et al., 2013 [9]	Mouse model of chronic and binge ethanol feeding (the NIAAA model)	To demonstrate a model that induces liver damage using the Lieber-DeCarli model plus a single binge, which contributes to studies of alcohol consumption in the induction of diseases in the liver and other tissues.	679 (56,58)	729	948	10.1038/nprot.2013.032
2	Ki et al., 2010 [37]	Interleukin-22 Treatment Ameliorates Alcoholic Liver Injury in a Murine Model of Chronic-Binge Ethanol Feeding: Role off Signal Transducer and Activator of Transcription 3	To investigate how IL-22 can help in the pathogenesis of alcoholic liver injury caused by excessive alcohol consumption in animal models.	321 (21,4)	355	484	10.1002/hep.23837
3	Momen-Heravi et al., 2015 [38]	Exosomes derived from alcohol-treated hepatocytes horizontally transfer liver-specific miRNA-122 and sensitize monocytes to LPS	To examine how binge drinking significantly increases the number of exosomes in alcoholic hepatitis.	237 (23,7)	258	342	10.1038/srep09991
4	Bertola et al., 2013 [29]	Chronic Plus Binge Ethanol Feeding Synergistically Induces Neutrophil Infiltration and Liver Injury in Mice: A Critical Role for E-selectin	Significantly deepens the hepatic inflammation of the chronic and compulsive model of alcohol intake in rats and recorded that this model caused infiltration of hepatic neutrophils compared to other models of close consumption.	198 (28,29)	227	248	10.1002/hep.26419
5	Chao et al., 2018 [30]	Impaired TFEB-Mediated Lysosome Biogenesis and Autophagy Promote Chronic Ethanol-Induced Liver Injury and Steatosis in Mice	To investigate how binge drinking affects autophagy and lysosomal function by analyzing the TFEB factor that regulates lysosome biogenesis.	198 (16,5)	220	254	10.1053/j.gastro.2018.05.027
6	Ruhl et al., 2005 [40]	Joint effects of body weight and alcohol on elevated serum alanine aminotransferase in the United States population	To evaluate the relationship of alcohol consumption and binge drinking with an abnormality in serum aminotransferase activity in normal-weight and overweight individuals.	185 (9,25)	202	261	10.1016/s1542-3565(05)00743-3 3565(05)00743-3
7	DiMartini et al., 2006 [41]	Alcohol consumption patterns and predictors of use following liver transplantation for ALD	To investigate alcohol consumption in patients with ALD after liver transplantation.	180 (9,47)	216	281	10.1002/lt.20688
8	Hendrikx, et al., 2019 [42]	Bacteria engineered to produce IL-22 in the intestine induce expression of	Understanding the mechanism of REG3G suppression induced by	174 (29,0)	189	222	10.1136/gutjnl-2018-317232

		REG3G to reduce ethanol-induced liver disease in mice	chronic-plus-single-binge ethanol feeding.				
9	Heo et al., 2019 [43]	Alcohol dysregulates miR-148a in hepatocytes through FoxO1, facilitating pyroptosis via TXNIP overexpression	Evaluate hepatocyte-specific miRNAs altered by the progression of ALD through excessive alcohol consumption.	165 (27,5)	179	203	10.1136/gutjnl-2017-315123
10	Mansouri et al., 1999 [44]	An alcoholic binge causes massive degradation of hepatic mitochondrial DNA in mice	To investigate the effects of ethanol on the liver mitochondria of experimental animals and alcoholic individuals, with an emphasis on oxidative stress and mitochondrial DNA deletions.	159 (6,12)	169	209	10.1016/S0016-5085(99)70566-4
11	Li et al., 2017 [45]	MicroRNA-223 ameliorates alcoholic liver injury by inhibiting the IL-6-p47phox-oxidative stress pathway in neutrophils	To investigate the relationship between liver damage, linked to an increase in circulating neutrophils, and binge drinking, as well as to understand the miR-223 burden in neutrophil modulation.	157 (19,63)	180	208	10.1136/gutjnl-2016-311861
12	Khanova et al., 2018 [46]	Pyroptosis by caspase11/4-gasdermin-D pathway in alcoholic hepatitis in mice and patients	To understand the genetic profile of liver tissue from humans and animals with alcoholic hepatitis induced by binge drinking coupled with a high-fat diet and which genes are regulated in the two models.	148 (21,14)	164	181	10.1002/hep.29645
13	Ekstedt et al., 2009 [47]	Alcohol consumption is associated with progression of hepatic fibrosis in non-alcoholic fatty liver disease	To elucidate whether light alcohol intake in patients with non-alcoholic fatty liver disease can corroborate the development of liver fibrosis.	148 (9,25)	170	234	10.1080/00365520802555991
14	Abdelmegeedet al., 2013 [26]	CYP2E1 potentiates binge alcohol-induced gut leakiness, steatohepatitis, and apoptosis	To investigate the burden of compulsive ethanol consumption on intestinal CYP2E1 and its possible hepatic and enteric consequences.	143 (11,92)	149	191	10.1016/j.freeradbiomed.2013.09.009
15	Roerecke, et al., 2019 [48]	Alcohol Consumption and Risk of Liver Cirrhosis: A Systematic Review and Meta-Analysis	Elucidate the risks involved between various patterns of alcohol intake and the incidence of liver cirrhosis	141 (23,5)	161	237	10.14309/ajg.0000000000000340
16	Neyrinck et al., 2017 [49]	Rhubarb extract prevents hepatic inflammation induced by acute alcohol intake, an effect related to the modulation of the gut microbiota	Investigating the possible hepatoprotective mechanism of rhubarb in liver damage induced by binge drinking and its effects on the intestinal microbiota	132 (16,5)	151	184	10.1002/mnfr.201500899
17	Carson et al., 1996 [50]	Development and characterization of a binge drinking model in mice for evaluation of the immunological effects of ethanol	To develop a murine model of binge drinking for immunological evaluation, with emphasis on antibody responses.	130 (4,48)	138	192	10.1111/j.1530-0277.1996.tb01055.x

18	Chang et al., 2015 [51]	Short- or long-term high-fat diet feeding plus acute ethanol binge synergistically induce acute liver injury in mice: An important role for CXCL1	To demonstrate that the high-fat diet plus binge alcohol administration intensified liver injury, as well as the number of neutrophils in the liver.	128 (12,8)	148	173	10.1002/hep.27921
19	Ni et al., 2013 [52]	Critical Role of Foxo3a in Alcohol-Induced Autophagy and Hepatotoxicity	To analyse FoxO3a-regulated autophagy in the liver induced by episodic alcohol ingestion in the hypothesis that it is a mechanism for reducing microsteatosis and possible liver damage.	126 (10,5)	135	162	10.1016/j.ajpath.2013.08.011
20	Lee et al., 2017 [53]	Three-year Results of a Pilot Program in Early Liver Transplantation for Severe Alcoholic Hepatitis	To investigate a pilot protocol for early liver transplantation in selected patients with acute alcoholic hepatitis.	124 (15,5)	137	189	10.1097/SLA.0000000000001831
21	Williams et al., 2015 [54]	Parkin regulates mitophagy and mitochondrial function to protect against alcohol-induced liver injury and steatosis in mice	To analyze the hepatoprotective action of Parkin, due to its role in maintaining alcohol-affected mitochondria, against liver damage induced by compulsive alcohol consumption.	124 (12,4)	130	152	10.1152/ajpgi.00108.2015
22	Peng et al., 2016 [55]	A rationally designed rhodamine-based fluorescent probe for molecular imaging of peroxynitrite in live cells and tissues	To demonstrate the effectiveness of HKYellow, a fluorescent probe capable of detecting and recording peroxynitrite in different biological mechanisms.	123 (13,67)	127	126	10.1039/c6sc00012f
23	Wilsnack, et al., 2018 [56]	Gender Differences in Binge Drinking Prevalence, Predictors, and Consequences	To analyze the disparities and the impact of the different definitions of binge drinking, as well as the different research approaches and techniques, on the results obtained.	119 (17,0)	63	230	not available
24	Kim et al., 2006 [57]	Acute in vivo effect of ethanol (binge drinking) on histone H3 modifications in rat tissues	To understand the burden of binge drinking on the acetylation or methylation of histone H3 at lysine 9 in various rodent tissues	115 (6,05)	126	170	10.1093/alcalc/agh248
25	Demeilliers et al., 2002 [58]	Impaired adaptive resynthesis and prolonged depletion of hepatic mitochondrial DNA after repeated alcohol binges in mice	To investigate the effect of binge drinking on hepatocyte mtDNA in a murine model.	115 (5,0)	134	173	10.1053/gast.2002.35952
26	Zhou et al., 2001 [30]	Ethanol-induced apoptosis in mouse liver - Fas- and cytochrome c-mediated caspase-3 activation pathway	Investigate the relationship between upstream signals, FAS, and cytochrome C in the activation of caspase-3, induced hepatic apoptosis, and binge drinking of ethanol.	112 (4,67)	122	166	10.1016/S0002-9440(10)61699-9
27	Cahill et al., 2002 [59]	Effects of alcohol and oxidative stress on liver pathology: The role of the mitochondrion	Investigate the relationship between upstream signals, FAS, and cytochrome C in the activation of caspase-3, induced	111 (4,83)	119	160	10.1111/j.1530-0277.2002.tb02621.x

			hepatic apoptosis, and binge drinking of ethanol.				
28	Moon et al., 2006 [60]	Inactivation of oxidized and S-nitrosylated mitochondrial proteins in alcoholic fatty liver of rats	Investigating oxidized and/or S-nitrosylated mitochondrial proteins and understanding inactivation in alcoholic fatty livers in a murine model.	109 (5,74)	115	133	10.1002/hep.21372
29	Ramirez et al., 2017 [61]	Aging aggravates alcoholic liver injury and fibrosis in mice by downregulating sirtuin 1 expression	To examine the impacts of aging on alcohol-induced liver damage, and the burden of sirtuin 1 (SIRT1) and microRNA 223 (miR-223) in the pathogenesis of ALD.	106 (13,25)	120	144	10.1016/j.jhep.2016.11.004
30	Parker et al., 2018 [62]	Alcohol, adipose tissue and liver disease: mechanistic links and clinical considerations	Investigating the effects of alcohol on adipose tissue and the connection between the drug, the liver, and adipose tissue	105 (15,0)	115	157	10.1038/nrgastro.2017.116
31	Cui et al., 2015 [63]	Invariant NKT cells promote alcohol-induced steatohepatitis through interleukin-1β in mice	To investigate the action of interleukin in liver injury activated by binge drinking intake that generates neutrophil infiltration and consequently inflammation.	103 (10,3)	114	136	10.1016/j.jhep.2014.12.027
32	Yang et al., 2014 [33]	Cannabidiol protects liver from binge alcohol-induced steatosis by mechanisms including inhibition of oxidative stress and increase in autophagy	To evaluate the hepatoprotective effect of cannabidiol on hepatic steatosis induced by oxidative imbalance derived from binge drinking.	102 (9,27)	112	153	10.1016/j.freeradbiomed.2013.12.026
33	Xu et al., 2015 [64]	Fat-Specific Protein 27/CIDEC Promotes Development of Alcoholic Steatohepatitis in Mice and Humans	To identify molecules linked to the progression of alcoholic steatohepatitis, with emphasis on FSP-27 and CIDEC, which are responsible for inducing fat droplets in adipose tissue.	99 (9,9)	113	143	10.1053/j.gastro.2015.06.009
34	Mathurin et al., 2009 [65]	Effect of binge drinking on the liver: an alarming public health issue?	To demonstrate the possible hepatic repercussions induced by compulsive alcohol consumption.	99 (6,19)	108	143	10.1136/gut.2007.145573
35	Roh et al., 2015 [66]	TLR2 and TLR9 contribute to alcohol-mediated liver injury through induction of CXCL1 and neutrophil infiltration	Investigating the burden of TLR2 and TLR9 signaling on hepatic neutrophil infiltration in binge drinking	98 (9,8)	104	139	10.1152/ajpgi.00031.2015
36	Chu et al., 2020 [67]	The Candida albicans exotoxin candidalysin promotes alcohol-associated liver disease	To evaluate the possible burden of Candida albicans and its exotoxin, candidalysin, on ALD.	96 (19,2)	111	138	10.1016/j.jhep.2019.09.029
37	Mathews et al., 2014 [68]	Animals Models of Gastrointestinal and Liver Diseases. Animal models of alcohol-induced liver disease:	To discuss models of ALD in mice, with emphasis on the chronic plus binge ethanol feeding model.	95 (8,64)	112	139	10.1152/ajpgi.00041.2014

		pathophysiology, translational relevance, and challenges					
38	Cresci et al., 2017 [69]	Prophylactic tributyrin treatment mitigates chronic-binge ethanol-induced intestinal barrier and liver injury	Investigate the prophylactic effect of tributyrin and its potential protection of the intestinal and liver layer of mice exposed to excessive alcohol consumption.	93 (11,63)	105	127	10.1111/jgh.13731
39	Bukong et al., 2018 [70]	Abnormal neutrophil traps and impaired efferocytosis contribute to liver injury and sepsis severity after binge alcohol use	To evaluate the formation of NETs and their role in the clearance of sepsis by macrophages after excessive alcohol intake. The authors state that neutrophil dysfunction increases the liver damage associated with sepsis.	92 (13,14)	103	111	10.1016/j.jhep.2018.07.005
40	Zhou et al., 2018 [71]	Neutrophil-Hepatic Stellate Cell Interactions Promote Fibrosis in Experimental Steatohepatitis	Investigating the role of neutrophils in the progression of steatohepatitis induced by binge drinking	92 (13,14)	97	126	10.1016/j.jcmgh.2018.01.003
41	Desai et al., 2017 [72]	Fibroblast growth factor 21 (FGF21) is robustly induced by ethanol and has a protective role in ethanol associated liver injury	To examine the performance of FGF21 in response to alcohol consumption in humans and mice. This research reproduced repeated episodes of binge drinking to identify FGF21 as a therapeutic target for alcoholic liver disease.	90 (11,25)	94	129	10.1016/j.molmet.2017.08.004
42	Massey et al., 2012 [73]	Acute alcohol-induced liver injury	Understanding the burden of PAI-1 and fibrin metabolism in the treatment of acute liver injury caused by alcohol consumption	88 (6,77)	103	145	10.3389/fphys.2012.00193
43	Dastidar et al., 2018 [74]	Rodent Models of ALD: Role of Binge Ethanol Administration	Compile positive points, restrictions, and scope of different models of liver diseases, caused by alcohol consumption.	86 (12,29)	87	115	10.3390/biom8010003
44	Fuster et al., 2018 [75]	Alcohol Use in Patients with Chronic Liver Disease	To explore the consequences of excessive alcohol consumption on different liver manifestations and the therapeutic approach of individuals with ALD.	84 (12,0)	117	185	10.1056/NEJMra1715733
45	Chen et al., 2016 [76]	*Lactobacillus rhamnosus* GG supernatant promotes intestinal barrier function, balances Treg and TH17 cells and ameliorates hepatic injury in a mouse model of chronic-binge alcohol feeding	Discuss the repercussions of moderate ethanol consumption on cardiovascular and liver diseases, in addition to non-alcoholic fatty liver disease.	84 (9,33)	90	101	10.1016/j.toxlet.2015.11.019.

46	Chen et al., 2015 [77]	Microbiota Protects Mice Against Acute Alcohol-Induced Liver Injury	To evaluate the role of the intestinal microbiota in liver damage induced by excessive alcohol consumption.	84 (8,4)	90	180	10.1111/acecr.12900
47	Lazaro et al., 2015 [78]	Osteopontin Deficiency Does Not Prevent but Promotes Alcoholic Neutrophilic Hepatitis in Mice	Investigate the effects of osteopontin on ALD induced by a high in cholesterol and saturated fat diet plus alcohol plus weekly binge ethanol.	84 (8,4)	90	116	10.1002/hep.27383
48	Aberg et al., 2020 [79]	Risks of Light and Moderate Alcohol Use in Fatty Liver Disease: Follow-Up of Population Cohorts	Discuss the effects of alcohol consumption on non-alcoholic fatty liver disease and other illnesses.	83 (16,6)	92	125	10.1002/hep.30864
49	Ajmera et al., 2017 [80]	Is Moderate Alcohol Use in Nonalcoholic Fatty Liver Disease Good or Bad? A Critical Review	Emphasising the consequences of moderate alcohol consumption on non-ALD, the levelling of alcoholic and non-ALD and establish the future studies needed to better understand this situation.	83 (13,83)	92	129	10.1002/hep.29055
50	Cresci et al., 2014 [81]	Tributyrin Supplementation Protects Mice from Acute Ethanol-Induced Gut Injury	To evaluate the repercussions of the prophylactic use of tributyrin on intestinal and liver damage induced by excessive alcohol intake.	83 (10,38)	89	115	10.1111/acer.12428
51	Hyun et al., 2021 [35]	Pathophysiological Aspects of Alcohol Metabolism in the Liver	Review studies on ethanol metabolism in the liver with the aim of securing data on the progression of ALD.	83 (7,55)	92	135	10.3390/ijms22115717
52	Wang et al., 2019 [82]	n-3 Polyunsaturated fatty acids for the management of ALD: A critical review	Discuss the burden of n-3 polyunsaturated fatty acids (n-3 PUFAs) in ALD, induced by excessive alcohol consumption, in a murine model	82 (20,05)	85	116	10.1080/10408398.2018.1544542
53	Nassir et al., 2014 [83]	Role of mitochondria in ALD	Investigate therapeutic possibilities for the treatment of ALD in humans, analyzing the functioning of biogenesis and the action of mitochondria interfered with ethanol.	80 (7,27)	95	147	10.3748/wjg.v20.i9.2136
54	Lamas-Paz et al., 2018 [84]	ALD: Utility of animal models	To review experimental models of alcohol consumption that induce liver disease, as well as to understand the mechanisms involved in ALD.	79 (11,29)	91	142	10.3748/wjg.v24.i45.5063
55	Carmiel-Haggai et al., 2003 [85]	Binge ethanol exposure increases liver injury in obese rats	To determine the hepatic repercussions of binge drinking in genetically obese rodents.	79 (7,59)	82	117	10.1053/j.gastro.2003.09.019
55	Lowe et al., 2017 [86]	Alcohol-related changes in the intestinal microbiome influence neutrophil	To examine enteric microbiome changes in murine alcoholic steatohepatitis induced by excessive alcohol	78 (9,75)	84	116	10.1371/journal.pone.0174544

		infiltration, inflammation and steatosis in early alcoholic hepatitis in mice	consumption. In addition to understanding the burden of intestinal microorganisms in alcoholic liver pathologies.				
56	Hatton et al., 2009 [87]	Drinking patterns, dependency and life-time drinking history in alcohol-related liver disease	To investigate the burden of excessive alcohol consumption on the increase in deaths caused by liver disease in the United Kingdom.	77 (4,81)	84	139	10.1111/j.1360-0443.2008.02493.x
57	Yin et al., 2007 [88]	Differential gene expression and lipid metabolism in fatty liver induced by acute ethanol treatment in mice	To analyze intrahepatic gene expression in rodents exposed to excessive alcohol consumption.	77 (4,28)	81	108	10.1016/j.taap.2007.06.018
58	Aberg et al., 2017 [89]	Binge drinking and the risk of liver events: A population-based cohort study	To analyze the hypothesis of an increased risk of liver disease due to binge drinking, with a considerable risk from moderate alcohol consumption.	76 (9,5)	82	103	10.1111/liv.13408
59	Mandrekar et al., 2016 [90]	Alcoholic Hepatitis: Translational Approaches to Develop Targeted Therapies	To bring together translational studies on the pathogenesis of alcoholic hepatitis that have not met the demanded needs, preclinical tools, and therapeutic targets with the aim of helping people with alcoholic hepatitis	75 (8,33)	88	102	10.1002/hep.28530
60	Saha et al., 2015 [91]	Alcohol-Induced miR-27a Regulates Differentiation and M2 Macrophage Polarization of Normal Human Monocytes	Establish the regulatory effect of miR-27 on the stimulation and polarization of leukocytes caused by alcohol consumption	75 (7,5)	81	102	10.4049/jimmunol.1402190
56	Lehwald et al., 2012 [92]	β-Catenin Regulates Hepatic Mitochondrial Function and Energy Balance in Mice	Elucidate the role of β-catenin in the process of cellular respiration or in the mitochondria of liver cells	75 (5,77)	75	91	10.1053/j.gastro.2012.05.048
57	Li et al., 2018 [93]	Liver kinase B1/AMP-activated protein kinase-mediated regulation by gentiopicroside ameliorates P2X7 receptor-dependent alcoholic hepatosteatosis	To evaluate the role of gentiopicroside in regulating the production of NLRP3 inflammasomes by the P2Z7 receptor in alcoholic hepatosteatosis.	72 (10,29)	76	82	10.1111/bph.14145
58	Zhao et al., 2015 [94]	FGF21 mediates alcohol-induced adipose tissue lipolysis by activation of systemic release of catecholamine in mice	To investigate the action of FGF21 in regulating adipose tissue lipolysis associated with compulsive alcohol consumption, after exposing murines to ethanol and to perceive intracellular changes and activation of lipolytic enzymes.	72 (7,2)	74	99	10.1194/jlr.M058610
59	Zhou et al., 2014 [27]	Sulforaphane induces Nrf2 and protects against CYP2E1-dependent binge alcohol-induced liver steatosis	To investigate the potential effect of sulforaphane on hepatic steatosis	72 (6,55)	84	106	10.1016/j.bbagen.2013.09.018

			produced by excessive alcohol consumption.				
60	Wang et al., 2017 [95]	Cannabidiol attenuates alcohol-induced liver steatosis, metabolic dysregulation, inflammation and neutrophil-mediated injury	Investigate the effects of cannabidiol on liver damage caused by chronic and compulsive ethanol consumption in rodents	71 (8,88)	92	147	10.1038/s41598-017-10924-8
61	Wu et al., 2012 [25]	Alcohol steatosis and cytotoxicity: The role of cytochrome P4502E1 and autophagy	Understand the role of CYP2E1 in alcoholic steatohepatitis, and consequently in the autophagy process, oxidative imbalance, and fat accumulation in liver tissue, induced by excessive alcohol consumption.	71 (5,46)	81	109	10.1016/j.freeradbiomed.2012.07.005
62	Bai et al., 2016 [96]	Betulin alleviated ethanol-induced alcoholic liver injury via SIRT1/AMPK signaling pathway	To evaluate betulin and its possible hepatoprotective effect on steatosis and alcoholic liver damage induced by compulsive alcohol consumption, via modulation of SIRT1-LKB1-AMPK	70 (7,78)	92	93	10.1016/j.phrs.2015.12.022
63	Iracheta-Vellve et al., 2018 [97]	FXR and TGR5 Agonists Ameliorate Liver Injury, Steatosis, and Inflammation After Binge or Prolonged Alcohol Feeding in Mice	Investigate new therapeutic targets to treat early-stage alcoholic steatohepatitis in mice - farnesoid X receptor and Takeda G protein agonists.	69 (9,86)	74	86	10.1002/hep4.1256
64	Cai et al., 2017 [98]	Mitochondrial DNA-enriched microparticles promote acute-on-chronic alcoholic neutrophilia and hepatotoxicity	To analyze the levels of exosomes in different patterns of alcohol consumption that induce inflammation by increasing circulating neutrophils + increasing mitochondrial DNA in microparticles due to oxidative stress, and consequent liver damage.	69 (8,63)	74	86	10.1172/jci.insight.92634
65	Stein et al., 2016 [99]	Heavy daily alcohol intake at the population level predicts the weight of alcohol in cirrhosis burden worldwide	To investigate the relevance of the daily use of alcoholic beverages in the contribution of determinants to the population risk of cirrhosis in the world.	68 (7,56)	75	108	10.1016/j.jhep.2016.06.018
66	Denaes et al., 2016 [100]	The Cannabinoid Receptor 2 Protects Against ALD Via a Macrophage Autophagy-Dependent Pathway	To explore the underlying action and mechanisms involved in the protective effect of the CB2 receptor on liver resident macrophages in alcohol-induced liver damage.	68 (7,56)	70	85	10.1038/srep28806
67	Yang et al., 2012 [32]	Cytochrome P4502E1, oxidative stress, JNK, and autophagy in acute alcohol-induced fatty liver	To examine the possibility of CYP2E1 playing a role in acute hepatic steatosis caused by ethanol. Excessive alcohol consumption can induce CYP2E1 and JNK activation, and autophagy.	67 (5,15)	71	86	10.1016/j.freeradbiomed.2012.06.029

68	Yang et al., 2003 [101]	Ethyl pyruvate ameliorates acute alcohol-induced liver injury and inflammation in mice	To determine whether delayed therapy with ethyl Ringer's pyruvate is efficient in treating liver damage induced by excessive alcohol consumption in a murine model.	67 (3,05)	74	104	10.1016/S0022-2143(03)00138-0
69	Zahr et al., 2010 [102]	Brain Injury and Recovery Following Binge Ethanol: Evidence from In Vivo Magnetic Resonance Spectroscopy	Examine neurological lesions after excessive use of ethanol, and complement the study by assessing whether there have been liver changes.	66 (4,4)	70	92	10.1016/j.biopsych.2009.10.028
70	Lim et al., 2014 [103]	Relationship Between Alcohol Use Categories and Noninvasive Markers of Advanced Hepatic Fibrosis in HIV-Infected, Chronic Hepatitis C Virus-Infected, and Uninfected Patients	To analyze the relationship between alcohol consumption models and severe liver fibrosis.	65 (5,91)	69	92	10.1093/cid/ciu097
71	Gao et al, 2015 [104]	Anti-inflammatory function of ginsenoside Rg1 on alcoholic hepatitis through glucocorticoid receptor related nuclear factor-kappa B pathway	To investigate the anti-inflammatory and hepatoprotective role of Rg1 in liver damage due to excessive alcohol use.	64 (6,4)	72	81	10.1016/j.jep.2015.07.020
72	Maricic et al., 2015 [105]	Inhibition of Type I Natural Killer T Cells by Retinoids or Following Sulfatide-Mediated Activation of Type II Natural Killer T Cells Attenuates ALD in Mice	To determine the influence of NKT cells on inflammation in ALD induced by chronic-plus-single-binge ethanol feeding.	64 (6,4)	72	82	10.1002/hep.27632
73	Zhong et al., 2014 [106]	Acute Ethanol Causes Hepatic Mitochondrial Depolarization in Mice: Role of Ethanol Metabolism	To demonstrate how mitochondrial depolarisation and the repercussions of ethanol and other drug metabolism affect steatosis and liver damage.	63 (5,73)	52	66	10.1371/journal.pone.0091308
74	Robin et al., 2005 [107]	Alcohol increases tumor necrosis factor α and decreases nuclear factor-κB to activate hepatic apoptosis in genetically obese mice	To evaluate the repercussions of compulsive alcohol use in lean and obese rodents and the mechanisms that limit the progression of oxidative stress.	63 (3,15)	69	83	10.1002/hep.20949
75	Wang et al., 2016 [108]	Increased hepatic receptor interacting protein kinase 3 expression due to impaired proteasomal functions contributes to alcohol-induced steatosis and liver injury	To analyze how excessive ethanol intake impacts RIP3 kinase and its accumulation in the induction of hepatic steatosis and necroptosis.	62 (6,89)	71	90	10.18632/oncotarget.6893
75	Wetterling et al., 1999 [109]	Drinking pattern and alcohol-related medical disorders	Evaluate the relationship between alcohol consumption patterns and health problems in alcoholic individuals.	62 (2,38)	77	137	10.1093/alcalc/34.3.330
76	Chen et al., 2018 [110]	DEP domain-containing mTOR-interacting protein suppresses lipogenesis and ameliorates hepatic	To examine the development of hepatic steatosis caused by excessive alcohol consumption, using the chronic-plus-binge ethanol feeding model in rodents.	61 (8,71)	68	9	10.1002/hep.29849

		steatosis and acute-on-chronic liver injury in ALD					
77	Mathews et al., 2016 [111]	Invariant natural killer T cells contribute to chronic-plus-binge ethanol-mediated liver injury by promoting hepatic neutrophil infiltration	To analyse how NKT cells act in the hepatic inflammatory process when there is a large number of neutrophils, caused by the administration of an excessive dose of ethanol in an animal model.	61 (6,78)	69	81	10.1038/cmi.2015.06
77	Cho et al., 2017 [112]	Increased Ethanol-Inducible Cytochrome P450-2E1 and Cytochrome P450 Isoforms in Exosomes of Alcohol-Exposed Rodents and Patients With Alcoholism Through Oxidative and Endoplasmic Reticulum Stress	To examine whether oxidative stress induced by excessive alcohol increases the number of CYP2E1 in exosomes of murine and human patients, as well as to test the possibility of CYP2E1 being a specific biomarker of alcohol exposure when compared to liver damage induced by other chemical compounds.	60 (7,5)	69	89	10.1002/hep4.1066
78	Shukla et al., 2013 [113]	Binge Ethanol and Liver: New Molecular Developments	To bring together the latest studies on the relevance of molecular research into the impact of alcohol on the development of therapies that seek to understand and improve the lives of patients with ALD.	60 (5,0)	70	86	10.1111/acer.12011
79	Lee et al., 2020 [114]	Mitochondrial Double-Stranded RNA in Exosome Promotes Interleukin-17 Production Through Toll-Like Receptor 3 in Alcohol-associated Liver Injury	To evaluate the weight of liver double-stranded mtRNA in ALD caused by compulsive alcohol consumption, as well as to demonstrate therapeutic mechanisms in the activation of IL-1β in macrophages.	58 (11,6)	59	82	10.1002/hep.31041
80	Mitchell et al., 2018 [115]	Type and Pattern of Alcohol Consumption is Associated With Liver Fibrosis in Patients With Non-alcoholic Fatty Liver Disease	To investigate whether the liver fibrosis found in patients with non-alcoholic fatty liver disease is associated with the amount, pattern of binge drinking or type of alcohol.	58 (8,29)	60	78	10.1038/s41395-018-0133-5
81	Liu et al., 2014 [116]	Luteolin Alleviates ALD Induced by Chronic and Binge Ethanol Feeding in Mice	To investigate the role of luteolin as a therapeutic target for ALD caused by binge drinking.	57 (5,18)	63	77	10.3945/jn.114.193128
82	Cho et al., 2017 [117]	Increased liver-specific proteins in circulating extracellular vesicles as potential biomarkers for drug- and alcohol-induced liver injury	To investigate the potential of circulating extracellular vesicles as an indicator of liver damage resulting from binge drinking and the use of paracetamol.	56 (7,0)	60	81	10.1371/journal.pone.0172463
83	Byun et al., 2013 [118]	Activation of toll-like receptor 3 attenuates alcoholic liver injury by	To examine the repercussions of TLR3 activation by macrophages and hepatic	56 (4,67)	63	89	10.1016/j.jhep.2012.09.016

		stimulating Kupffer cells and stellate cells to produce interleukin-10 in mice	stellate cells on the development of liver damage induced by compulsive ethanol use.				
84	Seberg et al., 2018 [119]	FGF21, a liver hormone that inhibits alcohol intake in mice, increases in human circulation after acute alcohol ingestion and sustained binge drinking at Oktoberfest	To investigate whether plasma levels of FGF21 in humans increase acutely or subchronically in response to alcohol consumption, considering the association between genetic variants in the KLB gene and levels of alcohol intake.	55 (7,86)	57	77	10.1016/j.molmet.2018.03.010
85	Varga et al., 2018 [120]	β-Caryophyllene protects against alcoholic steatohepatitis by attenuating inflammation and metabolic dysregulation in mice	To investigate the therapeutic effects of β-caryophyllene (BCP) in a model of chronic liver injury induced by binge drinking, analyzing its anti-inflammatory capacity and exploring the possible influence of activation of CB2 cannabinoid receptors on its mechanism of action.	54 (7,71)	65	88	10.1111/bph.13722
94	Sim et al., 2015 [121]	L-Serine Supplementation Attenuates Alcoholic Fatty Liver by Enhancing Homocysteine Metabolism in Mice and Rats	To examine the influence of L-serine on the development of alcoholic hepatic steatosis caused by binge drinking or a standard diet containing ethanol.	54 (5,4)	57	72	10.3945/jn.114.199711
95	Kirpich et al., 2012 [122]	Binge Alcohol-Induced Microvesicular Liver Steatosis and Injury are Associated with Down-Regulation of Hepatic Hdac 1, 7, 9, 10, 11 and Up-Regulation of Hdac 3	To examine the impact of binge drinking on the expression of histone deacetylase RNAs in the liver and how this expression affects the development of liver changes.	54 (4,15)	59	66	10.1111/j.1530-0277.2012.01751.x
96	Moro-Sibilot et al., 2016 [123]	Mouse and Human Liver Contain Immunoglobulin A-Secreting Cells Originating From Peyer's Patches and Directed Against Intestinal Antigens	To investigate how liver B cells produce immunoglobulin A (IgA) in response to binge drinking, both in mice and in human liver samples.	52 (5,78)	62	88	10.1053/j.gastro.2016.04.014
97	McCuskey et al., 2005 [125]	Ethanol hinging exacerbates sinusoidal endothelial and parenchymal injury elicited by acetaminophen	To investigate the effects of binge drinking on sinusoidal endothelial cells and hepatocytes and whether they become more sensitive to paracetamol toxicity.	52 (2,6)	63	71	10.1016/j.jhep.2004.11.033
98	Wang et al., 2018 [126]	Inflammation Is Independent of Steatosis in a Murine Model of Steatohepatitis	To investigate the role of peroxisome proliferator-activated receptor gamma (PPARγ) in the regulation of steatosis and neutrophil infiltration induced by binge drinking associated with a high-fat diet.	51 (6,38)	50	64	10.1002/hep.29129

99	Kai et al., 2020 [127]	Oroxylin a promotes PGC-1α/Mfn2 signaling to attenuate hepatocyte pyroptosis via blocking mitochondrial ROS in ALD	Investigating the role of oroxilin A in liver pyroapoptosis induced by binge drinking	50 (10,0)	57	57	10.1016/j.freeradbiomed.2020.03.031
100	Cho et al., 2018 [128]	Apoptosis of enterocytes and nitration of junctional complex proteins promote alcohol-induced gut leakiness and liver injury	To examine how programmed cell death of intestinal cells and the process of chemical modification of specific proteins contribute to the increase in intestinal permeability caused by binge drinking.	50 (7,14)	53	65	10.1016/j.jhep.2018.02.005

ALD: alcoholic liver disease; ROS: reactive oxygen species; PGC-1α/Mfn2: coactivator 1 alpha/ mitofusin 2; PPARγ: PPARα = peroxisome proliferator-activated receptor alpha; Hdac: Hepatic histone deacetylase; FGF21: Fibroblast growth factor 21; RNA: ribonucleic acid; miRNAs: MicroRNAs; TFEB: transcription factor EB; REG3G: Antimicrobial C-type lectin regenerating islet-derived 3 gamma; IL-22: Interleukin 22; FoxO1: forkhead box protein O1; TXNIP: thioredoxin-interacting protein; IL-6: Interleukin 6; CYP2E1: cytochrome P450 2E1; CXCL1: (C-X-C motif) ligand 1; mtDNA: mitochondrial DNA; SIRT1: sirtuin 1; miR-223: microRNA 223; NKT: natural killer T; FSP-27: fat-specific protein 27; TLR: Toll-like receptor; FGF21: Fibroblast growth factor 21; TH17: T helper 17 cells; n-3 PUFAs: n-3 polyunsaturated fatty acids; miR-27A: microRNA 27a; NLRs: NOD-like receptors; FXR: farnesoid X receptor; TGR5: Takeda G protein-coupled receptor 5; CB2: Cannabinoid Receptor 2; RIP3: receptor-interacting protein kinas; TLR-3: toll-like receptor 3; PGC-1α/Mfn2: peroxisome proliferator-activated receptor gamma coactivator 1 alpha; PPARγ: peroxisome proliferator-activated receptor gamma; IgA: immunoglobulin A; BCP: β-caryophyllene.

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3.3. Authors' Contribution

A total of 603 authors contributed to at least one of the selected articles (as shown in Figure 4A). Among the authors with the highest number of publications, we highlight Gao B, with 19 articles, which is 2.25 times more than the second author with the most publications, Xu MJ, who presents 9 articles. In the sequence appear the Pacher P and Wang H authors, both with 7 articles each (as shown in Figure 4C). Furthermore, when examining the co-authorship network, we see that Gao B is the most connected to other authors, i.e. he has the main network of collaborative connections (as shown in Figure 4B). In terms of the number of citations, the authors who stand out are Gao B, Wang H, and Bertola A, with 2,660, 1,239, and 1,199 citations, respectively. It is interesting to note that, although Bertola has not contributed as many articles as Gao B, his work is widely cited by other researchers.

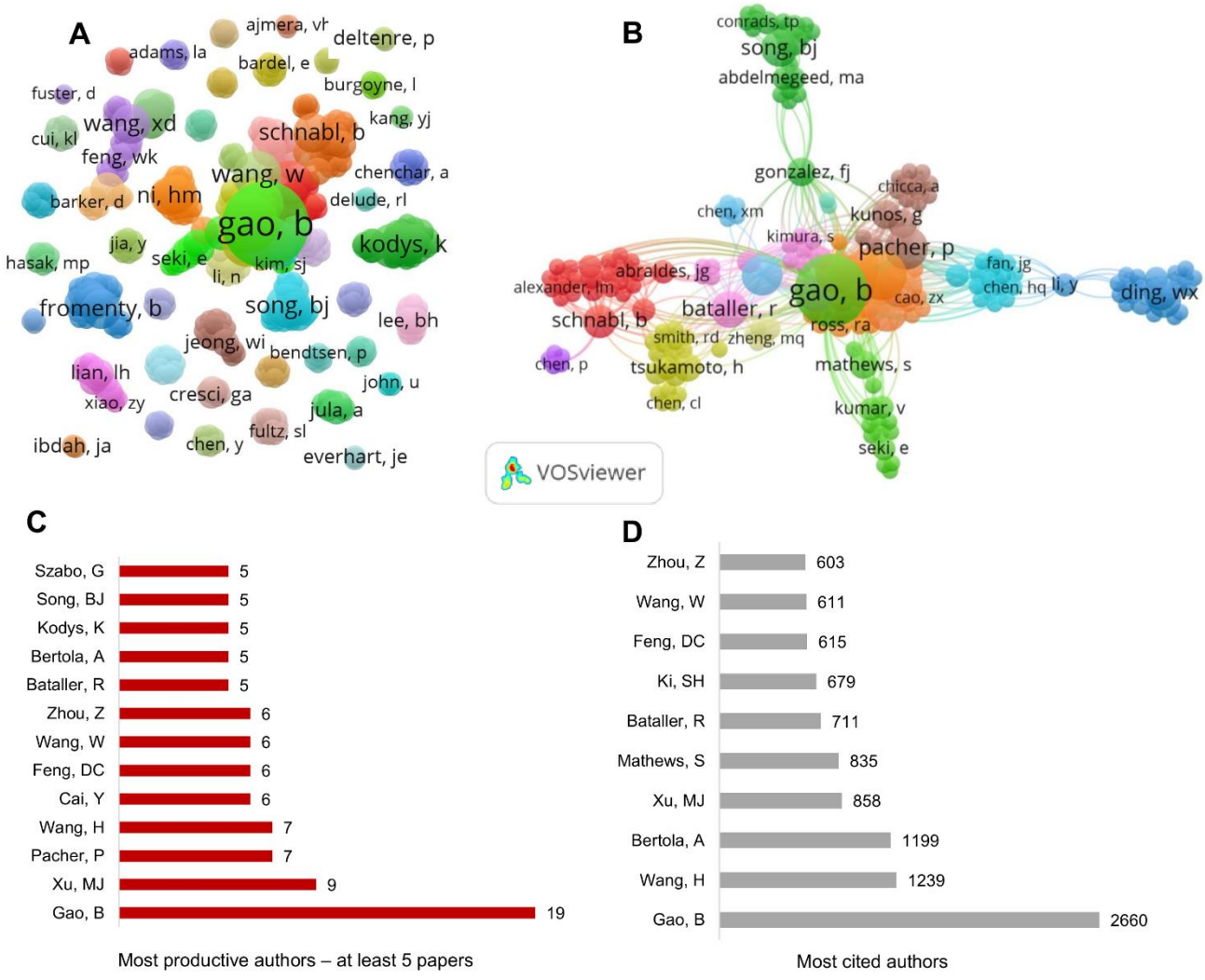

Figure 4. Visualization of the network of authors with the number of publications (A), main network (B), authors with the highest number of publications (C), and most cited authors (D).

Keywords

A total of 160 keywords were identified by the authors in a single occurrence (Figure 5A). The 10 most used terms were "Alcohol" (n=21), "Alcoholic Liver Disease" (n=15), "Liver Steatosis" (n=14), "Binge Drinking" (n=10), "Inflammation" (n=7), "Liver" (n=6), "Oxidative Stress" (n=6), "CYP2E1" (n=5), "Steatohepatitis" (n=5) and "Autophagy" (n=4) (Figure 5 B). When we evaluate the network associated with the term "Binge Drinking", the most linked terms are: "Alcohol", "Oxidative Stress", "Liver", "Free Radicals", "CYP2E1", "Autophagy", "Liver Injury", "Nuclear Factor Erythroid 2-Rel", "Liver Steatosis", "Fibroblast Growth Factor 21" "Alcoholic Liver Disease", "High Fat Diet", "Histones Deacetylases", "Chronic ethanol consumption", "Neutrophil Depletion", "Alcoholic Hepatitis", "Immune System", "Animal Model", "Acute Ethanol", "Apoptosis" and "Cirrhosis". This network can show us the themes addressed in the scientific field regarding episodic and intermittent alcohol consumption versus liver disorders. It should also be noted that, in recent years, the most common terms used include "intestinal balance", "microbiota", "intestinal barrier", "intestinal permeability", "neutrophil depletion", "fibroblast growth factor 21", "lipotoxicity", "high-fat diet", "gene regulation", "liver protection", "alcohol metabolism" and "alcohol-related liver disease" (Figure 5B).

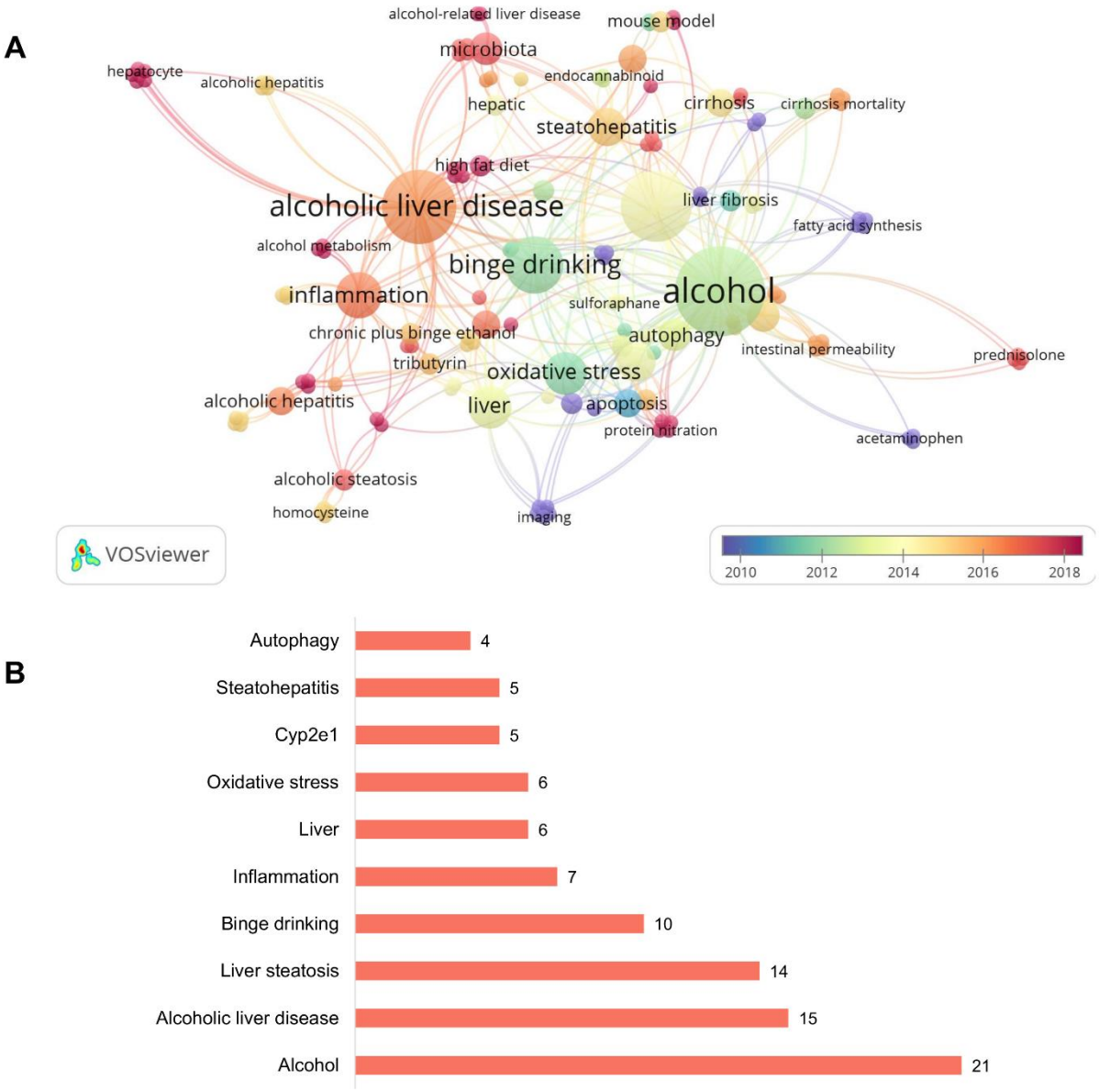

Figure 5. Keyword network selected by the authors. The size of the cluster shows the frequency of keyword use and the lines show the connection between them (A). The most frequently terms used are shown in the figure (B).

Journals

Only the journals with more than two articles published are shown in Figure 6; however, the full register of all journals and their respective impact factors (IF) (Table 2, Supplementary Information). The journal with the highest number of publications was "Hepatology" (IF: 14.0), with 14 published documents, followed by "Journal of Hepatology" (IF: 25.7) with 8 articles,

"Gastroenterology" with 7 publications (IF: 29.4) and "Alcoholism-Clinical and Experimental Research", with 6 published works (Figure 6).

Figure 6. Journals with the highest number of publications and their impact factors. Vertical bar representation that visualizes the distribution of scientific publications in different journals. Each bar represents a specific journal, and the length of the bar indicates the number of publications in that journal.

It was also observed that, among the journals analyzed, the New England Journal of Medicine stood out for having the highest impact factor, with an impressive 158.5, which indicates its great influence in the academic world. In comparison, the Nature Protocol, although published the most cited article [9], presents an impact factor of 14.8, which is considered highly respectable and qualified in the academic field.

Publication Period

Between 2011 and 2021, there was the highest number of articles published on the subject, totaling 80, with a peak in 2018, when 16 documents were published (Figure 7A). This period was

marked by a wide variety of study types. Although it was a promising period, no selected article was published in 2011. The second period with the highest number of publications was between 2001 and 2010, with 17 articles, especially 2005, 2006, and 2009, which published 3 documents per year. On the other hand, only 3 articles were published between 1996 and 1999, representing the shortest period of time and number of publications. This shortening is justified by the interval between the oldest article selected and the most recent (Figure 7A). Among the types of research recorded, during the period from 1996 to 1999, *in vivo* laboratory articles received the highest number of citations (n=289), followed by observational studies (n=62). From 2001 to 2010, *in vivo* experimental studies were the most cited documents (n=1176), followed by observational studies (n=510), and reviews (n=210). In the decade from 2011 to 2021, *in vivo* experimental studies stood out in terms of the number of citations (n=4,060), followed by reviews (n=1,118) and *in vivo/in vitro* experimental studies (n=669) (Figure 7B).

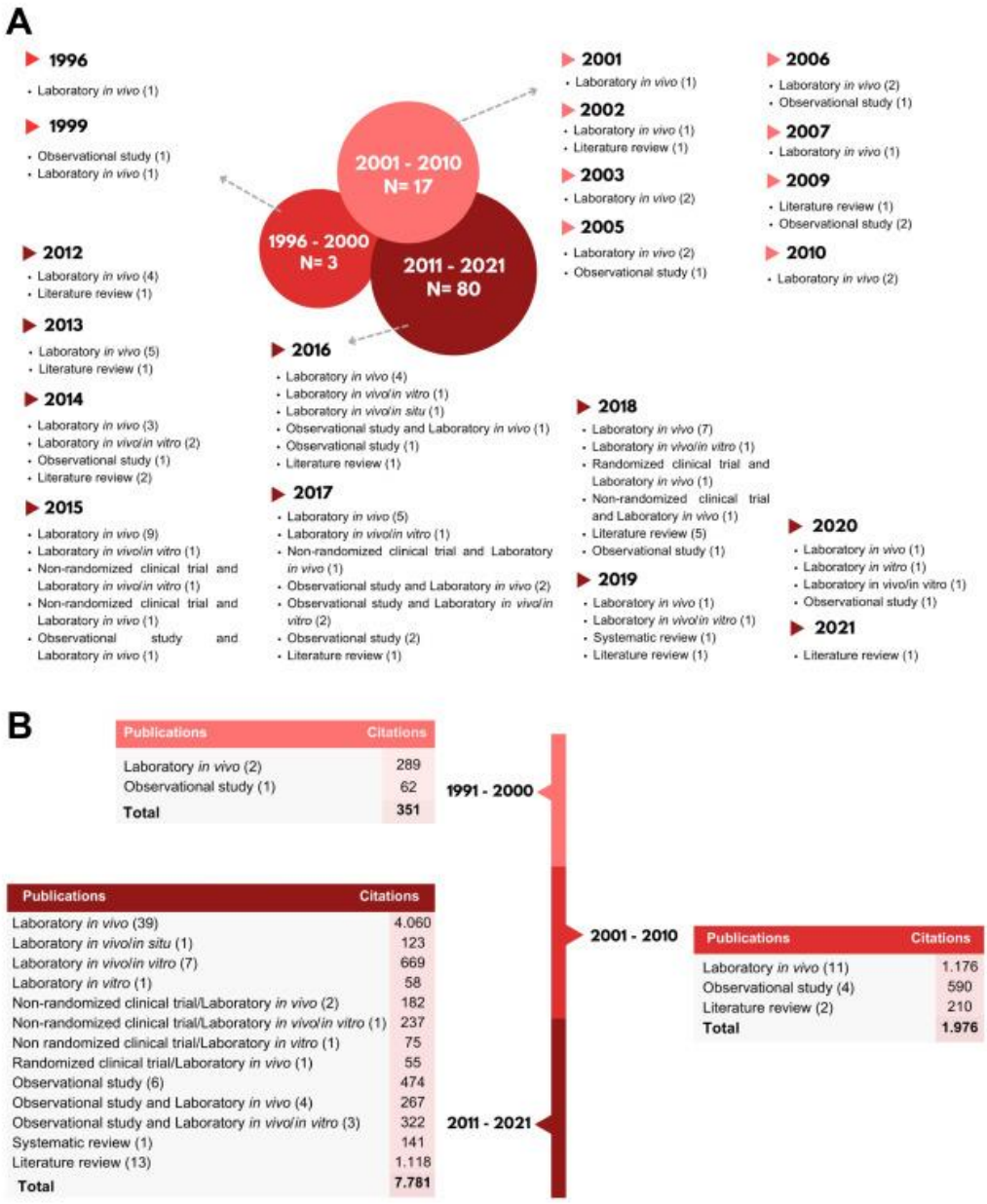

Figure 7. Overview of study types over the years. Historical and quantitative series of the types of study published (A) and the corresponding number of citations (B).

Countries' Contribution

The United States of America stands out in the number of publications, since of the 100 articles selected, 69 are by American corresponding authors, followed by China with 10 articles and South Korea and France with 6 articles each (Figure 8A; Figure 8B). This trend is repeated when analyzing

the number of citations, in which the USA accounts for 7,618 of the total number of citations, followed by China, with 772 mentions, and France, with a total of 556 citations (Figure 8C).

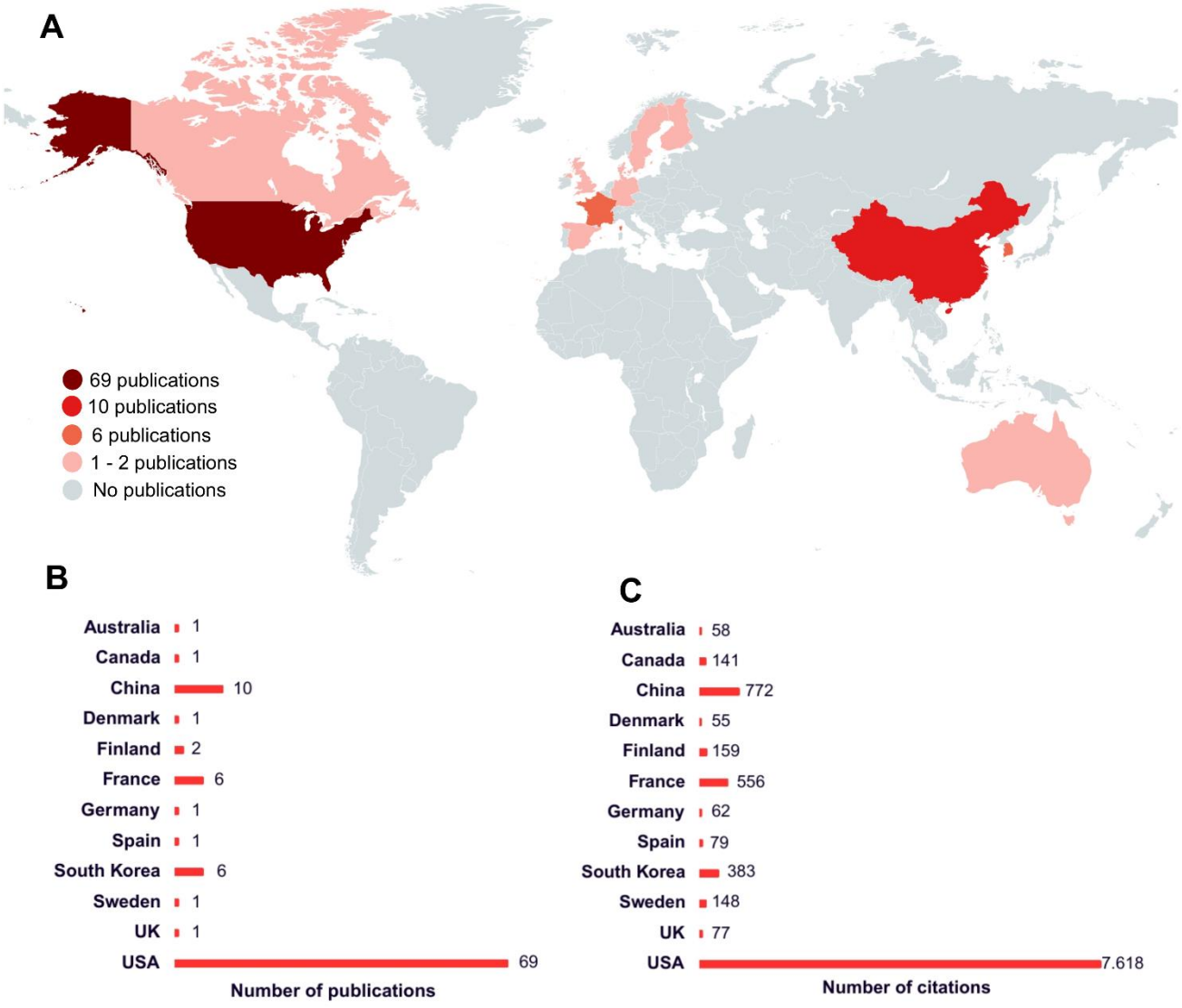

Figure 8. Geographical and numerical visualization of publications and citations of selected articles. Global distribution of the selected documents (A), with representation of the countries where they were published (B) and the total number of citations received (C).

Funding Agencies

In terms of funding agencies, the National Institutes of Health (NIH) stands out for providing direct support to 60 of the 100 selected articles. This number does not include indirect funding from its institutes and centers, which could further increase the total. Additionally, the National Institute on

Alcohol Abuse and Alcoholism (NIAAA), a division of NIH, also played a crucial role, funding 45 of the selected articles (Table 3, Supplementary Information).

These two institutions are the largest supporters of studies on binge drinking and its associations with liver alterations, as evidenced by their status as the main funders of the five most cited articles in our selection (Figure 9). When analyzing the 10 most cited articles and their respective funding sources, we identified a similar pattern: seven were funded by the NIH and five specifically by the NIAAA, with all these studies conducted in the United States. Although South Korea has increased its academic output on this topic, none of the articles from this country among the 10 most cited received financial support from either the NIH or the NIAAA (Figure 9). Despite being a U.S. government agency, the NIH has also supported research conducted in other countries. Lastly, it is worth noting that, among the 100 selected articles, 14 authors did not disclose their funding sources (Table 3, Supplementary Information).

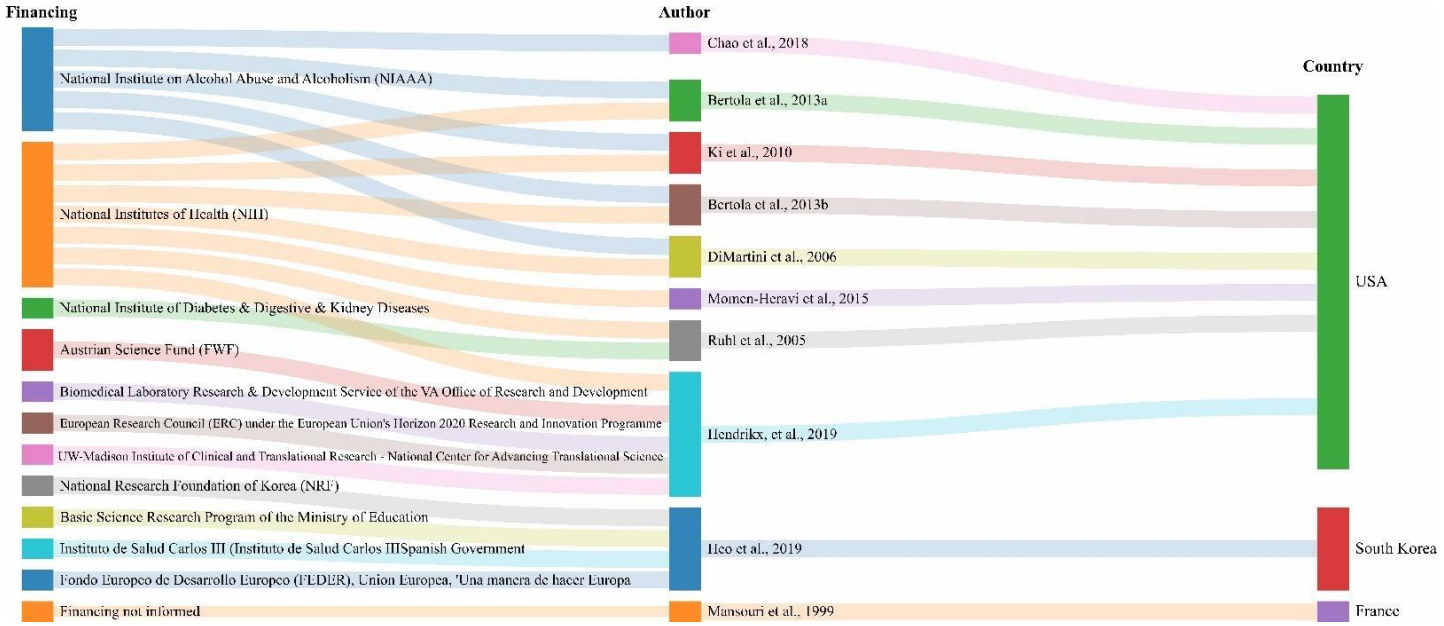

Figure 9. Sankey diagram of the 10 most cited articles, their respective funding organizations, and countries. The figure shows the relationship between sources of funding for research and the countries where the research was carried out. The horizontal lines connect each source of funding to the corresponding country, indicating that a particular piece of research funded by a specific source was carried out in that country.

Experimental Studies

Understanding the mechanisms by which binge drinking contributes to liver damage is essential, not only to provide information on the pathophysiology of these conditions but also to guide prevention and intervention strategies. In this context, 71 experimental studies were analyzed, and their

data was extracted for the construction of the knowledge mapping. *In vivo* experimental articles that applied ethanol binge-like protocols in animal models were included. Among these documents, five distinct ethanol binge-like protocols were identified: “Ethanol binge”, “Chronic diet plus ethanol single-binge”, “Ethanol binge + acetaminophen”, “High cholesterol diet and saturated fat alcohol (HCFD) + alcohol + weekly binge” and “High-fat diet (HFD) + ethanol binge”. It is important to note that more than one protocol was often evaluated in the same study.

An analysis of the articles evaluating a single protocol shows that the “binge-like ethanol” method is the most frequently used, reported in a total of 35 studies. However, it is important to note that, despite sharing the same nomenclature, these studies show variations in the ethanol administration regime. These variations include single, intermittent, and episodic administrations. All of these regimes have been classified by the authors themselves as “binge ethanol”.

Another widely used method is “Chronic plus binge ethanol feeding “(with a total of 30 studies), which consists of feeding an alcoholic diet for several days or months, followed by a single or multiples episodes of binge like ethanol.

All the protocols were applied to murine models (mice and rats), with an emphasis on the C56BL/7 strain of mice, which was the most widely used in most of the studies. The doses of alcohol administered ranged from 1 to 7 g/kg of body weight, with the most common dose being 5g/kg. It is important to note that all the studies exposed the animals to alcohol through intragastric administration, except for two studies that also evaluated the combination of intraperitoneal and intragastric administrations, as well as a comparison between the two routes of administration.

The table 3 shows the main results relating to liver parameters, considering the genetic variations in the animals or different exposure protocols. In cases where both parameters were addressed, priority was given to data from wild animals and to the full exposure protocols carried out.

Table 3. Knowledge mapping of documents that used experimental "binge drinking" protocols and their hepatic effects.

	Title	Binge Protocol / Binge Pattern				Key Results of Protocol	Summary
		Binge type	Dose/Route	Period	Model/Gender/Age		
1	Mouse model of chronic and binge ethanol feeding (the NIAAA model)	Chronic-plus-single-binge ethanol feeding	Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/6 male mice / 56 – 70 days old C57BL/6 male mice / 70 – 84 days old	- Increased AST and ALT - Infiltration of neutrophils	This document describes a simple model for inducing liver damage by means of the Lieber-DeCarli alcoholic diet, followed by a binge or multiple binges. When applied, these protocols produce inflammation, steatosis and liver damage. These models of liver disease induction also allow the investigation of alcohol consumption in other tissues.
2	Interleukin-22 Treatment Ameliorates Alcoholic Liver Injury in a Murine Model of Chronic-Binge Ethanol Feeding: Role off Signal Transducer and Activator of Transcription 3	Chronic-plus-single-binge ethanol feeding	Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/6 male mice / 56 – 70 days old	- Increase in ALT, AST and hepatic triglycerides; - Increased markers: CCR2, F4/80, IFN- γ, IL-6 and MCP-1; - Reduction in hepatic GSH and increase in 4-HNE; - Increased expression of SREBP-1, FAS, LXRα, ACC and SCD1 - Reduced expression of PPARα	This study induced alcoholic liver injury in animals to evaluate how IL-22 can improve the pathogenesis of the disease induced by binge drinking. The protocol applied produced liver damage, inflammation and oxidative imbalance, as well as increased expression of genes responsible for lipogenesis and reduced expression of genes that activate fat oxidation. The use of IL-22 reduced alcoholic liver damage, possibly due to its antioxidant, antiapoptotic, proliferative and antimicrobial activities.
3	Exosomes derived from alcohol-treated hepatocytes horizontally transfer liver specific miRNA-122 and sensitize monocytes to LPS	Binge-like ethanol	5g/kg of ethanol 50% (v/v), intragastric rout	Single intragastric administration of ethanol.	C57BL6/J female mice/ 42- 56 days old	- Increase in miRNA-22 in Kupffer cells and liver monocytes; - Increase in pro-inflammatory cytokines; - Increase in exosomes in the blood circulation	The authors of this article intend to investigate the loading of exosomes in alcoholic hepatitis, induced by binge drinking. In addition to understanding the possible relationship between these vesicles and immune cells. The protocol applied increased the number of exosomes in the circulation and identified a possible regulatory role of exosomes in communication between hepatocytes and monocytes, as well as inducing sensitivity to LPS through miRNA-122.
4	Chronic Plus Binge Ethanol	Chronic-plus-single-binge		Chronic ethanol feeding for 10 days, ad libitum	C57BL / 6J and SELE −/− female	**Chronic-plus-single-binge ethanol feeding:**	This study evaluated the hepatic alterations resulting from different

	Feeding Synergistically Induces Neutrophil Infiltration and Liver Injury in Mice: A Critical Role for E-selectin	ethanol feeding; binge-like ethanol	Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route Binge ethanol: 5 g kg, intragastric route	and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	mice / 56-70 days old	- Increased ALT and AST - Increase in the number of neutrophils infiltrating the liver parenchyma; - Positive regulation of pro-inflammatory cytokines; **Binge-like ethanol:** - Decreased macrophage labeling and unchanged levels of monocyte detection - Small but significant increase in ALT and AST.	patterns of alcohol consumption, with an emphasis on inflammatory research. The results obtained show that the alcohol binge protocols induced liver damage and inflammation, which was subsequently attenuated by E-selectin.
5	Impaired TFEB-Mediated Lysosome Biogenesis and Autophagy Promote Chronic Ethanol-Induced Liver Injury and Steatosis in Mice	Chronic-plus-single-binge ethanol feeding	Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	GFP-LC3 transgenic mice, TFE3 KO and C57BL/6N male mice / 56 – 70 days old	**GFP-LC3 transgenic:** - Increase in GFP-LC3 associated with the autophagosomal membrane (puncta); - Reduction in total and nuclear TBEF in the liver; - increased translocation of mTOR and activation of mTORC1 in liver cells; **TFEB KO mice:** - Increase in ALT e triglyceride; **C57BL/6N male:** - Increase in ALT e triglyceride;	This work investigated the burden of alterations in autophagy and lysosomal functions induced by binge drinking in alcoholic liver disease. Binge drinking promotes steatosis and liver injury by reducing EB transcription factor expression and therefore inhibiting cellular protection mechanisms such as autophagy and lysosomal biogenesis.
6	Bacteria engineered to produce IL-22 in the intestine induce expression of REG3G to reduce ethanol-induced liver disease in mice	Chronic-plus-single-binge ethanol feeding	Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/6 and Reg3g –/– female and male mice / 56 – 84 days old	**C57BL/6 mice:** - Increased ALT and triglycerides; - Bacterial translocation to the liver **Reg3g –/– mice:** - Increased ALT and triglycerides; - Bacterial translocation to the liver	The authors of this article investigated the mechanisms involved in the dysbiosis caused by binge drinking and the burden of this alteration on liver disease. The findings of this study show that intestinal dysbiosis, by impairing IL-22 production and intestinal REG3G expression, ends up increasing the translocation of bacteria to the liver and the development of steatohepatitis. Therefore, these findings could lead to studies to improve understanding of the liver-gut axis.

7	Alcohol dysregulates miR-148a in hepatocytes through FoxO1, facilitating pyroptosis via TXNIP overexpression	Binge-like ethanol	5 g/kg, intragastric route	Intragastric administration of ethanol twice daily for 7 days.	C57BL/6 male mice/ 42- 56 days old	- Reduction in hepatic miR-148a; - Increase in Txnip mRNA; - Decreased expression of Fox01 - Increased expression of NLRP3.	This study investigated the miRNAs present in hepatocytes altered by alcoholic liver disease. The present findings demonstrate that binge drinking induces hepatic inflammation and the progression of liver disease through the activation of the inflammasome.
8	An alcoholic binge causes massive degradation of hepatic mitochondrial DNA in mice	Binge-like ethanol	5 g kg, intragastric route	Single intragastric administration of ethanol.	CD-1(ICR)BR Swiss male mice/age not informed	- Decrease in hepatic mtDNA; - Degradation of hepatic mtDNA;	This research investigated binge drinking concerning structural alterations in mitochondrial DNA. The results of this study showed that animals intoxicated with ethanol had a consistent disruption of mitochondrial DNA in the liver.
9	MicroRNA-223 ameliorates alcoholic liver injury by inhibiting the IL-6-p47phox-oxidative stress pathway in neutrophils	Chronic-plus-single-binge ethanol feeding	Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	**Short-term:** Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol. **Long-term:** Ethanol liquid diet for 8 weeks, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet, plus multiple compulsions, with intragastric administration - twice a week during chronic feeding;	C57BL / 6J, miR-223 -/- and p47 phox - / - female mice / 70 – 84 days old	**C57BL / 6J mice:** - Increased AST and ALT; - Increase in MDA and 4-HNE; - Increase in pro-inflammatory mediators; - Positive regulation of hepatic and peripheral neutrophils; - Increased neutrophil infiltration **miR-223 -/- mice:** - Marked Increased AST and ALT; - Low triglyceride levels; - Higher levels of IL-6 - Increased neutrophil infiltration - Marked reduction in the number of F4/80 + Kupffer cells/macrophages; - Marked increase in the hepatic expression of inflammatory mediators; - Significant increase in malonaldehyde and 4-hydroxynonenal **p47 phox - / - mice:** - Reduction in ALT levels;	The authors of this study investigated the relationship between neutrophils and binge drinking. The protocol carried out produced oxidative imbalance, liver inflammation and hepatocyte damage. This study investigated miR-223 in-depth, one of the microRNAs most commonly found in neutrophils. Therefore, this RNA may be an important therapeutic target in alcoholic liver disease, as it can negatively modulate neutrophil infiltration.

						- Increase in hepatic neutrophils;	
10	Pyroptosis by caspase11/4-gasdermin-D pathway in alcoholic hepatitis in mice and patients	High in cholesterol and saturated fat diet (HCFD) + Binge- like ethanol	4 - 5g / kg by intragastric gavage	High cholesterol and saturated fat diet (HCFD), orally, ad deliberum, for two weeks. Then, intragastric feeding with alcohol or liquid diet rich in 60% fat, supplemented with HCFD diet, ad libitum, for 8 weeks. Plus intragastric administration of binge alcohol (4 to 5g/kg), weekly.	C57BL/6j, *Casp11* KO E IL-18 KO male mice / age not informed	**C57BL/6j mice:** - Activation of GSDMD; - Positive regulation of IL-17 mRNA in the liver; **Casp11 KO mice:** - Change in GSDMD activation; - Reduction in hepatic bacterial load; - Downregulation of IL-17 mRNA; **IL-18 KO mice:** - GSDMD activation; - Increase in hepatic bacterial load	This study aimed to investigate and compare the genetic profile of liver tissue from humans and animals that developed alcoholic hepatitis due to binge drinking associated with a high-fat diet. The results showed that the CASP11-GSDMD-pyroptosis pathway is fundamental in the development of alcoholic hepatitis in a murine model, and is also activated in humans who develop AH.
11	CYP2E1 potentiates binge alcohol-induced gut leakiness, steatohepatitis, and apoptosis	Binge-like ethanol	6g/kg, by intragastric gavage	Three intragastric administrations, 12 hours apart.	129/SvJ e Cyp2e1-null female mice // age not informed	**129/SvJ mice:** - Intense production of microvesicular intracellular lipid droplets; - Increase in hepatic triglycerides; - Deposition of neutrophils - Increased mRNA levels of inflammatory proteins - Significant increase in enterobacteria colonies in fresh liver extracts - Increased CYP2E1 activity and lipid peroxidation. - Marked uptake of pro-apoptotic proteins **Cyp2e1-null mice:** - Increase in triglycerides; - Increase in phosphorylated p-AMPK PPAR-α	This work evaluated the burden of intestinal CYP2E1 in the development of liver inflammation as a result of binge drinking and which mechanisms are involved in this process. It also sought to understand the pharmacological role of chlormethiazole and N-acetyl-cysteine in hepatic steatosis and endotoxemia. The results show that binge drinking can produce hepatic steatosis, tissue inflammation due to oxidative imbalance, systemic endotoxemia and increased hepatocyte apoptosis.
12	Rhubarb extract prevents hepatic inflammation induced by acute alcohol intake, an	Binge-like ethanol	6g/kg, by intragastric gavage	Single intragastric administration of ethanol.	C57BL/6J male mice / 84 days old.	- Increased expression of pro-inflammatory markers - Mild hepatic steatosis - Increase in serum ALT	The aim of this study was to evaluate the mechanisms involved in the effects of rhubarb extract on alcohol-induced liver injury in a murine model and to relate them to the microbiota,

	effect related to the modulation of the gut microbiota						inflammation, liver disorders and the intestinal barrier. The results obtained show that the alcohol protocol was capable of generating steatosis and marked hepatic inflammation - the latter induced by bacterial translocation from the intestine and consequent stimulation of pro-inflammatory mediators.
13	Development and characterization of a binge-drinking model in mice for evaluation of the immunological effects of ethanol	Binge-like ethanol	3,0–7,0 g/kg, by intragastric gavage	Single intragastric administration of ethanol.	C57B1/6 female mice / 56 and 112 days old	- ALT increased in the first analysis, after the 7g/kg binge drinking protocol; - ALT decreased at 24 hours after alcohol administration with 7g/kg; - Serum albumin and total protein decreased 4, 12 and 24 hours after the administration of 7g/kg of alcohol.	This study was designed to describe the progression and characterization of a model of episodic binge drinking. This protocol was used to evaluate the body's immune responses to toxic alcohol consumption. The dosages of alcohol used did not induce serious changes in chemical parameters. Only the 7g/kg dose was able to induce minor liver damage. It should be noted that the slight increase in ALT was not consistent with the levels observed in animals with developed liver damage. In addition, aminotransferase levels returned to 14normal shortly after alcohol administration.
14	Short- or long-term high-fat diet feeding plus acute ethanol binge synergistically induce acute liver injury in mice: An important role for CXCL1	High-fat diet (HFD) + ethanol binge model	Binge ethanol: 5g/kg, intragastric route	Feeding with HFD diet for 3 days, ad deliberum, orally. Followed by a single dose of ethanol intragastrically (short time). HFD diet for 3 months, ad deliberum, orally. Followed by a single dose of intragastric ethanol (long term)	C57BL/6 male mice / 56 – 84 days old	**HFD for 3 days + binge alcohol:** - Increase in AST and ALT; - Slight myeloperoxidase labeling of neutrophils; - Small 20-fold increase in Cxcl1 mRNA **HFD for 3 months + binge alcohol:** - Increase in ALT and AST; - Severe hepatic steatosis; - Intense myeloperoxidase labeling of neutrophils; - High labeling of inflammatory chemokines and adhesion molecules - especially CXCL1, which increased 30-fold.	The contributors to this article investigated how a high-fat diet provided for a long or short period, followed by alcohol administration, can intensify neutrophil infiltration in the liver and liver damage. The results of this study show that a high-fat diet plus alcohol administration induced hepatocyte damage, hepatic steatosis and intense tissue inflammation.

15	Critical Role of Foxo3a in Alcohol-Induced Autophagy and Hepatotoxicity	Binge-like ethanol	4,5 g/kg, intragastric route.	Accumulative dosage: four gavages at 15-minute intervals	C57BL/6 and Foxo3a −/− female and male mice	**C57BL/6 mice:** - Increased levels of mRNA and other proteins associated with autophagy - Increased expression of FoxO3a; - Increased nuclear translocation of Fox03a **Foxo3a −/− mice:** - Expression of autophagy-related genes was suppressed - Amount of autophagosomes was reduced - ALT and triglycerides increased	The authors of this study investigated the mechanisms involved in the autophagy process induced by episodic alcohol consumption and how this can reduce liver damage. The results demonstrate that alcohol consumption induces autophagy-mediated by FoxO3a, as a possible compensatory mechanism to reduce microesthetosis and damaged mitochondria, and consequently reduce liver damage.
16	Parkin regulates mitophagy and mitochondrial function to protect against alcohol-induced liver injury and steatosis in mice.	Chronic-plus-single-binge ethanol feeding; binge-like ethanol	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route Binge-like ethanol: 4,5 g/kg, intragastric rout	Chronic-plus-single-binge ethanol feeding: Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol. Binge-like ethanol: Four gavages at 15-minute intervals - cumulative dose, by gavage intragastric.	C57BL/6J and Parkin KO male mice / 56 – 84 days old	**HD:** - Increased ALT - Increase in CYP2E1 levels - Increase in triglycerides - Intense steatosis - Reduction of hepatic sterol regulatory element gene expression - Increased expression of genes involved in the β-oxidation pathway (Acox1 and Pparα) **Binge-like ethanol:** - Increased ALT - Increase in triglycerides - Mild steatosis - Reduction of hepatic sterol regulatory element gene expression	The present study investigated whether Parkin - recruited when mitochondria are damaged - protects liver tissue from alcohol-induced damage.
17	A rationally designed rhodamine-based fluorescent probe for molecular	Binge-like ethanol	5 g/kg, intragastric route	Accumulative dosage	Sprague–Dawley male rats / 56 – 84 days	- High generation of peroxynitrite in liver damage;	This study characterized a fluorescence probe called HKYellow, which allows the uptake of peroxynitrite in samples of living cells and tissues. In this work, analyzes were carried out on liver

	imaging of peroxynitrite in live cells and tissues					- High presence of peroxynitrite around the hepatic vasculature; - Increased tyrosine nitration	tissue previously exposed to excessive alcohol consumption. The results obtained demonstrate the first imaging evidence of increased tissue peroxynitrite in animals intoxicated by alcohol. That is, a major marker of oxidative damage developed during liver injury.
18	Acute in vivo effect of ethanol (binge drinking) on histone H3 modifications in rat tissues	Binge-like ethanol	6g/kg, intragastric Route	Single intragastric administration of ethanol.	Sprague–Dawley male rat / 56 days old	- Gradual increase in Ac-H3-LYS9; - Gradual reduction of hepatic Ac-H3-LYS9	The aim of this study is to understand and unravel the effects of binge drinking in vivo on the acetylation or methylation of histone H3 at lysine 9 in different rodent organs. In liver tissue, the results only highlight an increase in histone H3 methylation and selective post-translational acetylation of histone H3. These findings may contribute to alterations in the intoxicated liver through changes in DNA structure.
19	Impaired adaptive resynthesis and prolonged depletion of hepatic mitochondrial DNA after repeated alcohol binges in mice	Binge-like ethanol	6g/kg, intragastric route	Intragastric administration of ethanol once daily for 4 days.	Crl:CD-1(ICR)BR Swiss male mice	- Decrease in mtDNA at 2, 24 or 48 hours after the last administration of the 4 consecutive doses; - Significant increase in mtSSB liver transcripts 24 and 48 hours after the last intoxication; - Changes in mitochondrial structure 2 and 24 hours after the last administration; - Significant increase in TBARS 2 hours after the last dose of repeated intoxication; - Increased expression of CYP2E1	This study aimed to evaluate the effects of repeated alcohol intoxication on hepatic mtDNA in a murine model. The results show that repeated alcohol binges have the capacity to cause unrepaired damage to mitochondrial DNA, preventing its resynthesis and resulting in prolonged mitochondrial DNA depletion in the liver.
20	Ethanol-induced apoptosis in mouse liver - Fas- and cytochrome c-mediated caspase-3 activation pathway	Binge-like ethanol	6g/kg, intragastric route	Accumulative dosage: four gavages at 20-minute intervals.	FVB male mice / 70 days old	- Perivenous apoptosis; - DNA fragmentation; - Activation of caspase-3 in the nucleus and cytoplasm of perivenous hepatocytes; - Increase in cytosolic cytochrome in the liver.	This study aimed to understand the role of the Fas ligand and cytochrome C in the development of hepatic apoptosis observed in alcohol-intoxicated tissues. The results show that the alcohol protocol applied induced hepatocyte apoptosis via the caspase-3 activation pathway.

21	Inactivation of oxidized and S-nitrosylated mitochondrial proteins in alcoholic fatty liver of rats	Binge-like ethanol	5g/kg, intragastric route	Intragastric administration of ethanol once daily for 4 days.	Sprague-Dawley male rats / "young rats"	- Increase in nitrite levels; - Increased activity of NOS, iNOS and CYP2E1. - Hepatic steatosis	The results obtained show that binge drinking induced hepatic alterations due to increased oxidative and nitrosative stress, possibly as a consequence of the hyperactivity of some proteins, such as CYP2E1
22	Aging aggravates alcoholic liver injury and fibrosis in mice by downregulating sirtuin 1 expression	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Short term: Ethanol liquid diet for 10 days followed plus binge of ethanol. Long-term: Ethanol liquid diet for for 8 weeks, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet, plus multiple compusions, with intragastric administration - twice a week during chronic feeding	C57BL/6N female mice / young: 56 – 84; middle-aged: 84 – 98; old: >112.	**Short time:** - Greater increase in AST, ALT and triglycerides in middle-aged and older animals compared to older animals; - Greater accumulation of lipids in the liver tissue of middle-aged and older animals, when compared to older animals; - Higher levels of neutrophil infiltration in liver tissue in middle-aged animals compared to young animals. - Slight fibrosis in middle-aged animals. **Long term:** - Significant increase in AST, ALT and triglycerides in middle-aged animals compared to young animals; - Increase in apoptotic hepatocytes in middle-aged animals compared to young animals; Reduced liver regeneration in middle-aged animals compared to young animals; - More prominent marking of 4-HNE in liver tissue in middle-aged animals compared to young animals; - Fibrosis 4 to 5 times more pronounced in middle-aged mice when compared to young fed animals.	The authors of this study aimed to understand how binge drinking contributes to liver damage and its effects on tissue ageing. The results show that older animals are more susceptible to liver damage, estateatosis, tissue inflammation and oxidative stress when compared to younger animals exposed to binge drinking. These findings were observed in both the short-term and long-term protocols, showing greater intensity in the 8 weeks + multiple binge protocol.

23	Invariant NKT cells promote alcohol-induced steatohepatitis through interleukin-1β in mice	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic-plus-single-binge ethanol feeding: Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57Bl/6, Jα18-/-, Nlrp3-/- and IL-12p40-/- male mice / 42 – 56 days old.	**C57Bl/6 mice:** - Increased ALT and hepatic triglycerides; - Accumulation of NKT cells in liver tissue - Infiltration of neutrophils **Jα18-/- mice:** - Low levels of the hepatic cytokines TNF-α, IL-6 and IL-1β in the liver; - Reduction in the total number of neutrophils; - Little neutrophil activation - Low inflammation markers - Ly6G and E-selectin; **Nlrp3-/- mice:** - lower frequency and less activation of NKT cells; - Lower serum ALT levels; - Lower hepatic triglyceride levels - No change in the mRNA of components of the NLRP3 pathway **IL-12p40-/- mice:** - Accumulation of NKT cells in liver tissue	The authors of this study aimed to evaluate the role of IL-1B in steatohepatitis induced by binge drinking. The results show that binge drinking induced liver damage through neutrophil infiltration and consequent inflammation.
24	Cannabidiol protects liver from binge alcohol-induced steatosis by mechanisms including inhibition of oxidative stress and increase in autophagy	Binge-like ethanol	4 g/kg, intragastric Route	Intragastric administration of ethanol every 12 hours for 5 days.	C57Bl/6 female and male mice/ 56-70 old days	- Increase in ALT; - Decrease in ATP; - Lipid accumulation in the liver; - Intense labeling of 4-HNE and increase in ROS - Activation of the JNK pathway - Decreased LC3-II levels	The authors of the present study aimed to evaluate whether cannabidiol has a protective capacity in the liver with hepatic steatosis induced by binge drinking. The findings of this study show that binge drinking causes hepatic steatosis, possibly as a result of increased oxidative stress, reduced autophagy and activation of the JNK pathway, associated with apoptotic mechanisms. Therefore, hepatic alterations are promoted by multiple factors

25	Fat-Specific Protein 27/CIDEC Promotes Development of Alcoholic Steatohepatitis in Mice and Humans	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg or 6kg (Fsp27 Hep -/- mice), intragastric route	Binge- like ethanol: Single intragastric administration of ethanol. Short-term: Ethanol liquid diet for 10 days followed plus binge of ethanol. Long-term: Ethanol liquid diet for 8 weeks, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet, plus multiple compulsions, with intragastric administration - twice a week during chronic feeding Chronic plus-binge feeding: Ethanol liquid diet for 4, 8 or 12 weeks, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet, plus binge of ethanol	C57BL/6, Fsp27 Hep -/- male mice / 56 – 70 days old	**Short-term**: - Slight increase in AST and ALT; **Long-term**: - severe macrosteatosis; - Increase in AST and ALT; - Marked infiltration of neutrophils - Diffuse neutrophil infiltration; - Slight increase in 4-HNE levels in the liver - Increased expression of pro-inflammatory mediators **Chronic plus-binge ethanol feeding:** - Significant increase in AST and ALT; - Severe macrosteatosis; - Infiltration of agloremic neutrophils - Extensive increase in 4-HNE levels in liver tissue - Increased expression of pro-inflammatory mediators - Positive regulation of pre-keratin filament regulators;	This study applies different alcohol consumption protocols to experimental animals in order to identify molecules involved in the development of steatohepatitis. The findings show that models of chronic consumption followed by one or multiple binges, modify more than 1000 genes similar to those altered in alcoholic liver disease.
26	TLR2 and TLR9 contribute to alcohol-mediated liver injury through induction of CXCL1 and neutrophil infiltration	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic-plus-single-binge ethanol feeding: Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/6 female mice / 56 – 70 days old	- Increase in CXCL1 proteins - Neutrophil infiltration - Increase ALT; - Increase in pro-inflammatory cytokines	his study aimed to elucidate the role of TLR2 and TLR9 in liver injury and neutrophil infiltration, as well as to understand the role of CXCL1 in the pathogenesis of liver disease induced by alcoholic diet and/or ethanol binge in mice. The results show that the binge eating protocol used produced tissue damage through liver inflammation and hepatocyte death. In addition to hepatic steatosis. These results suggest that TLR2 and TLR9 signaling can induce liver damage through CXCL activation.
27	The Candida albicans exotoxin candidalysin	Chronic-plus-single-binge	Chronic-plus-single-binge ethanol feeding: Lieber-	Chronic-plus-single-binge ethanol feeding: Chronic ethanol feeding for 15	C57BL/6 and Clec7a −/− male	**C57BL/6 mice:** - Increase in ALT	This study evaluated the function of candidalisin, a cytotoxic peptide secreted by C. albicans, in animals

	promotes alcohol-associated liver disease	ethanol feeding	DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	mice / 56 – 84 days ols	- Accumulation of lipids in hepatocytes - Increase in inflammatory cytokines and chemokines **Clec7a −/−mice:** - Increase in triglycerides; - Increase in ALT; - Hepatic steatosis; - Increase in mRNA Il1b, Cxcl1, Cxcl2, Adh1 and Cyp2e1	submitted to a protocol that induces liver damage through binge drinking. The results of this study showed that the animals with alcoholic liver damage had liver damage, steatosis and inflammation in the tissue. When these same animals were colonized with C. albicans, these damage mechanisms were intensified. Therefore, demonstrating that candidalisin is an important author and a potential target in the pathogenesis of alcoholic liver disease.
28	Prophylactic tributyrin treatment mitigates chronic-binge ethanol-induced intestinal barrier and liver injury	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57Bl/6 female and male mice / 56 – 70 days old	- Increased expression of inflammatory cytokines (mRNA of TLR2, TLR4, TLR9); - Increase in inflammatory cytokines (TNFα) - Increase in ALT; - Apoptosis	This study aimed to investigate the function of tributyra in rodents with alcoholic hepatitis and subjected to a binge drinking protocol. This research aims to understand the protective role of tributyra in enteric integrity and whether it can somehow reduce the liver alterations caused by changes in the intestinal microbiota. It was observed that exposure to alcohol induced liver damage through mechanisms associated with inflammation, such as neutrophil infiltration and hepatocyte death.
29	Abnormal neutrophil traps and impaired efferocytosis contribute to liver injury and sepsis severity after binge alcohol use	Binge-like ethanol	5 mg / kg, intragastric route	Intragastric administration of ethanol once daily for 3 days.	C57BL/6J female mice / 70 – 94 days old	- Significant neutrophil infiltration 12 hours after LPS injection; - Excessive alcohol suppressed LPS-induced hepatic neutrophil extracellular traps (NET) formation; - Significant reduction in the induction of MCP-1 in the liver.	In this article, the authors explore the formation of NETs and their role in clearance by macrophages in sepsis following excessive alcohol consumption. For this, volunteers who drank alcoholic beverages and animals who also received alcohol and LPS were used. The authors report that neutrophil dysfunction and efferocytosis presented after alcohol consumption increase liver damage associated with sepsis. Therefore, demonstrating the important relationship that NETs have in patients with sepsis and alcohol consumption.
30	Neutrophil-Hepatic Stellate Cell Interactions	High-fat diet (HFD) +	Binge ethanol: 5g/ kg, intragastric route	HFD+1BD: HFD diet for 3 months, ad deliberum, orally.	C57BL/6J and Cxcl1 -/- male mice	**HFD+1BD mice:** - Increased expression of fibrosis-related genes;	The authors of this paper aimed to investigate the burden of neutrophil infiltration in the development of

	Promote Fibrosis in Experimental Steatohepatitis	ethanol binge model		Followed by a single dose of intragastric ethanol (long term) HFD+mB: HFD diet for 3 months, followed by a single binge or multiple binges (twice a week for a total of 8 times during an additional month)		- Marked increase in hepatic neutrophils - Intense 4-HNE labeling **HFD+mB mice:** - Intense increase in expression of fibrosis-related genes; - Slight increase in hepatic neutrophils;	steatohepatitis caused by a high-fat diet plus compulsive ethanol feeding. The HFD + 1 BD or multiple binge methodology induced severe liver damage and inflammation through the accumulation of neutrophils in liver tissue. They are also associated with the development of fibrosis.
31	Fibroblast growth factor 21 (FGF21) is robustly induced by ethanol and has a protective role in ethanol associated liver injury	Binge-like ethanol	3,5 g/kg, intragastric route; ethanol 20% (v/v), via intraperetoneal	Three intragastric administrations or an intraperitoneal injection	C57BL/6J female and male mice / 56 – 112 days old	- Increase in circulating FGF21 after administration of the first gavage of alcohol, with a peak 6 hours later; - Increase in the expression of FAS, SREBP-1c and SCD1 6 hours after the first administration of alcohol. - Reduction in PPARα marked at 3 and 6 hours after ethanol consumption	This study aims to evaluate the role of FGF21 in alcohol preference in humans and animals. The experiments used different alcohol consumption protocols to understand FGF21 as a therapeutic target in the treatment of alcoholic liver disease.
32	Lactobacillus rhamnosus GG supernatant promotes intestinal barrier function, balances Treg and TH17 cells and ameliorates hepatic injury in a mouse model of chronic-binge alcohol feeding	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/6 male mice / 70 days old	- Increased ALT and AST; - Macrosteatosis and microsteatosis; - Increase in E.coli bacteria in the liver; - Increase in liver damage markers (increase in TH17 and decrease in Treg); - Increase in neutrophil recruiting and regulatory cytokine (IL-17)	This study aimed to evaluate the effects of Lactobacillus rhamnosus GG on the differentiation process of T cells, specifically Th17 and Treg, in the pathogenesis of liver damage due to binge drinking. The results show liver damage due to neutrophil infiltration and increased proliferation of bacteria in the liver. In addition, they showed obvious macro- and microstatosis. These conditions were attenuated in animals that received joint administration of Lactobacillus rhamnosus GG.
33	Microbiota Protects Mice Against Acute Alcohol-Induced Liver Injury	Binge-like ethanol	3 g/kg, intragastric route	Single intragastric administration of ethanol.	C57BL/6 female mice / age not informed	- Increased ALT and triglycerides; - Infiltration of neutrophils	This research aimed to analyze the role of the intestinal microbiota in liver damage caused by binge drinking. The findings reveal that animals with an absent microbiota are more affected by

						- Increase in pro-inflammatory cytokines and chemokines - Increased hepatic expression of genes involved in fatty acid synthesis (SREBP-1, SCD-1, FASN and ACC-1) - Increased expressiochonn of the CYP2E1 gene and protein;	binge drinking, showing more pronounced liver damage when compared to animals with a normal microbiota. This damage develops as a result of inflammatory mechanisms and increased lipid synthesis.
34	Osteopontin Deficiency Does Not Prevent but Promotes Alcoholic Neutrophilic Hepatitis in Mice	High cholesterol and saturated fat diet (HCFD) + feeding of ethanol + binge-like ethanol	Binge ethanol: 4 a 5 g kg, intragastric route	High cholesterol and saturated fat diet (HCFD), orally, ad deliberum, for two weeks. Then, intragastric feeding with alcohol or liquid diet rich in 60% fat, supplemented with HCFD diet, ad libitum, for 8 weeks. Plus intragastric administration of binge alcohol (4 to 5g/kg), weekly.	C57BL/6J male mice / 56 days old	- Hepatomegaly; - Lipid accumulation; - Increased AST and ALT; - Increased parakeratin infiltration of inflammatory neutrophils, due to induction of MPO, CXCL1 and SPP1 - Positive regulation of fibrogenic genes;	This study analyzed the hepatic consequences of the Western diet rich in HCFD + intragastric feeding with alcoholic diet + weekly binge ethanol. The protocol adopted induced liver damage through hepatic inflammation, fibrosis, intense infiltration and impaired lipid metabolism. It also highlighted the role of SPP1 in the development of alcoholic liver disease
35	Tributyrin Supplementation Protects Mice from Acute Ethanol-Induced Gut Injury	Binge-like ethanol	5 g kg, intragastric route	Short-term EtOH: Oral administration of 1% ethanol, ad deliberum, for 2 days. Followed by 6% (v/v) ethanol for a further 2 days. Binge ethanol: Single intragastric administration of ethanol, 5 g/kg.	C57BL/6J female mice / 56 – 70 days old	**Short-term EtOH:** - Increase in ALT and triglycerides; '- Increased expression of inflammatory cytokines (TNFα and MCP1) **Binge ethanol:** - Increase in ALT and triglycerides - Increase in pro-inflammatory markers (TNFα, IL1β and MIP2)	The authors of this study evaluated the efficacy of tributyrin in liver and intestinal changes induced by binge drinking. Alcohol consumption induced liver inflammation, which was reversed by the use of tributyrin, with the exception of triglyceride levels. It also generated intestinal protection by maintaining the enteric epithelial barrier
36	Binge ethanol exposure increases liver injury in obese rats	Binge-like ethanol	4 g/kg, intragastric route	Intragastric administration of ethanol every 12 hours for 3 days.	Zucker male rat / 105 days old	- Increase in ALT; - Reduced antioxidant defenses in obese animals - Increased lipid peroxidation - Increase in CYP2E1 in both fa/fa and fa/? animals. - Increased caspase activity associated with apoptosis	In the present study, the authors investigated the effects of acute ethanol consumption on the liver of obese rats, reporting that binge drinking increased liver damage and apoptosis in obese rats compared to lean rats. Furthermore, they suggest that this damage may also lead to oxidative and nitrosative damage. These findings demonstrate that

							obesity and alcohol consumption are directly related to liver damage.
37	Alcohol-related changes in the intestinal microbiome influence neutrophil infiltration, inflammation and steatosis in early alcoholic hepatitis in mice	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/6 female mice / 48 – 56 days old	- Increased ALT and AST; - Increase in inflammatory biomarkers (TNFα, Cxcl1, MCP-1) - related to bacterial load; - Reduced expression of genes associated with lipid metabolism - Fat accumulation in hepatocytes	In this article, the authors investigate changes in the enteric microbiota in the face of alcoholic steatohepatitis. They report that acute or chronic alcoholic feeding has an important impact on the microflora in different aspects and consequently produces alterations in the liver, once through the binge drinking protocol applied, liver damage is observed as a result of inflammation and hepatic steatosis.
38	Differential gene expression and lipid metabolism in fatty liver induced by acute ethanol treatment in mice	Binge-like ethanol	0,05g or 5g/kg, intragastric route	Single intragastric administration of ethanol.	ICR male mice / age not informed	**Low dose:** - positive regulation of the SREBF1 gene in the L6 group **High dose:** - Positive regulation of *Srebf1*, *Acly*, *Fasn*, *Mod1* and *Scd1* in the High dose group 6 hours after; - Hepatic steatosis 24 hours after alcohol consumption;	This study investigated genes with differential expression that may serve as biomarkers to identify hepatic steatosis induced by binge drinking. The results obtained show that this pattern of consumption affects the expression of genes responsible for the synthesis of fatty acids, such as the targets of SREBP, which can consequently induce fatty liver.
39	β-Catenin Regulates Hepatic Mitochondrial Function and Energy Balance in Mice	Binge-like ethanol	5g/kg, intragastric route	Three intragastric administrations, 12 hours apart.	C57/Bl6 and β-catenina KO male mice / 56 – 84 days old	**C57/Bl6 mice:** Increase in reactive oxygen species; - Slight increase in ALT; - No steatosis **β-catenin KO mice:** - Intense increase in ALT; - Increase in triglycerides - Increased generation of reactive oxygen species - Microvesicular steatosis	In this article, the authors investigated the role of b-catenin in the hepatic metabolism of animals that received ethanol in a binge pattern. Ethanol binge drinking considerably increased hepatic steatosis and oxidative damage, especially in beta-catenin-deficient mice, suggesting that this protein plays a crucial role in mitochondrial dysfunctions that lead to liver disease
40	Liver kinase B1/AMP-activated protein kinase-mediated regulation by gentiopicroside ameliorates P2X7 receptor-dependent	Chronic-plus-single-binge ethanol feeding; binge-like ethanol	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic-plus-single-binge ethanol: Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/6 J female mice / 56 – 70 days old	**Chronic-plus-single-binge ethanol feeding:** -Increased ALT and AST (severe steatosis and inflammatory infiltrate); - Elevated triglycerides; - Increased lipogenesis;	The present study sought to understand the burden of NLRP3 inflammasome activation by gentiopicroside during alcoholic hepatosatosis induced by binge drinking. The binge-ethanol methodology induced acute hepatic steatosis by increasing SREBP1 activity and decreasing PPARα protein. In addition,

	alcoholic hepatosteatosis		Binge-like ethanol:	Binge-like ethanol: Every 12 hours for a total of three doses		- Negative regulation of genes responsible for lipid oxidation **Binge-like ethanol:** - Increased ALT and AST; - Increase in triglycerides; - Lipid accumulation in hepatocytes; - Increased lipogenesis - Increase in inflammatory cytokines	this protocol induced liver damage through inflammatory mechanisms. As for the chronic plus binge feeding protocol, the results were similar to the previous protocol, but with greater intensity, showing severe hepatic steatosis, inflammation and apoptosis. These results were mitigated by gentiopicroside.
41	FGF21 mediates alcohol-induced adipose tissue lipolysis by activation of systemic release of catecholamine in mice	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/6J and FGF21 KO male mice/ 56 – 70 days old	**C57BL/6J:** - Increased expression of FGF21 in the liver; - Reduction in adipocyte size; - Increase in hepatic triglyceride; - Increase in AST and ALT. **FGF21 KO:** - Increase in hepatic triglyceride; - Tendency to reduce AST and ALT.	In this article, the authors investigate the role of fibroblast growth factor (FGF) in regulating adipose tissue lipolysis related to excessive alcohol consumption. For this, animals exposed to alcohol were used to investigate the study proposal. The authors observed that after exposure the animals showed intracellular changes and activation of lipolytic enzymes. This is an attenuated factor compared to using FGF supplementation. Thus reducing the strong impact of alcohol consumption on the regulatory functions of adipose tissue.
42	Sulforaphane induces Nrf2 and protects against CYP2E1-dependent binge alcohol-induced liver steatosis	Binge-like ethanol	30% alcohol at a dose of 3g/kg, intragastric route	Intragastric administration of ethanol twice daily for 5 days..	SV129 and CYP2E1 KI male mice / age not informed	**SV192**: - No activation of Nrf2; - Significant increase in triglycerides; - Increase in activity - Oxidative imbalance - Increased CYP2E1 activity; - Reduction in hepatic LC3-II / LC3-I ratio **CYPE2E1 KI (only a few evaluations):** - Increased levels of activated Nrf2; - Increase in triglycerides;	In this article, the authors investigated the potential of sulforaphane, an Nrf2 activator, in reducing CYP2E1-dependent alcohol-induced steatosis. The protocol used induced oxidative stress, hepatic steatosis and impaired autophagy. According to the authors, after treatment with sulforaphane, there was a decrease in hepatic levels of ethanol, triglycerides and cholesterol, which was also observed in cell culture. Therefore, treatment with sulforaphane proved to be a potent target for reducing alcohol-induced hepatic steatosis.
43	Cannabidiol attenuates alcohol-induced liver	Chronic-plus-single-binge	Chronic-plus-single-binge ethanol feeding: Lieber-	Chronic-plus-single-binge ethanol: Chronic ethanol feeding for 10 days, ad	C57BL/6 J female mice / 70 – 96 days old	- Increased ALT and AST; - Increase in triglycerides;	This study investigated the effects of cannabidiol on liver damage caused by chronic binge drinking. The protocol

	steatosis, metabolic dysregulation, inflammation and neutrophil-mediated injury	ethanol feeding	DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.		- Increased mRNA expression of genes involved in fatty acid synthesis; - Reduced mRNA expression of genes involved in fat oxidation; - Accumulation of neutrophils; - Increased mRNA expression of reactive oxygen and nitrogen species	applied induced liver damage through oxidative/nitrosative stress, inflammatory mechanisms such as neutrophil infiltration and an increase in inflammatory cytokines. In addition to producing hepatic steatosis. This damage was attenuated by the use of cannabidiol, demonstrating that it could be an important therapeutic target in alcoholic liver disease.
44	Alcohol steatosis and cytotoxicity: The role of cytochrome P4502E1 and autophagy	Binge-like ethanol	3g/kg, intragastric route	Intragastric administration of 30% ethanol at a dose of 3g/kg twice a day for four consecutive days.	SV129, CYP2E1 KI and CYP2E1 KO male mice / age not informed	**SV129 mice:** - Elevated triglycerides; - Increase in CYP2E1 activity; - Reduction in GSH; - Increase in oxidative stress markers - Decrease in LC3 II / LC3 I ratio **CYP2E1 KO mice:** - No steatosis; - Undetectable CYP2E1 activity **CYP2E1 KI mice:** - Increase in the number of triglycerides; - Increase in CYP2E1 activity; - Reduction in GSH; - Increase in oxidative stress markers - Decrease in LC3 II / LC3 I ratio	This study sought to understand the role of CYP2E1 in modulating hepatic autophagy and other liver damage resulting from binge drinking. The findings of this study show that the protocol applied generated liver damage through impairment of macroautophagy, oxidative stress and hepatic steatosis, linked to increased catalytic activity of CYP2E1.
45	Betulin alleviated ethanol-induced alcoholic liver injury via SIRT1/AMPK signaling pathway	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic-plus-single-binge ethanol: Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/6 male mice / 56 days old	- Increased expression of SREBP-1; - Activation of HSCs - Increased expression of NF-κB; - Inhibition of AMPK activation; - Inhibition of SIRT1 in LX-2 cells;	This study sought to elucidate the mechanisms involved in the protective activity of Betulin against alcoholic liver injury induced by the chronic plus ethanol feeding protocol. Alcohol consumption induced liver damage through an imbalance in fatty acid synthesis and activation of the SIRT1-LKB1-AMPK signaling pathway.

						- Increased expression of fibronectin, collagen-I and α-SMA in LX-2 cells; - Increase in ALT and AST; - Increase in triglycerides	This damage was attenuated by treatment with Betulin.
46	FXR and TGR5 Agonists Ameliorate Liver Injury, Steatosis, and Inflammation After Binge or Prolonged Alcohol Feeding in Mice	Binge-like ethanol	Ethanol at 20% (v/v), intragastrically	Intragastric administration of ethanol twice daily for 4 days.	C57BL/6 female mice / 42 – 58 days old	- Increased ALT - Microvesicular steatosis; - No change in FASN expression; - Increased cleavage of caspases-1, a component of the inflammasome.	The authors of this work aimed to understand how FXR and/or TGR5 agonists can be used to treat alcoholic liver disease, induced by binge drinking. Our findings show that the binge-like ethanol protocol induced hepatic steatosis and acute inflammation.
47	Mitochondrial DNA-enriched microparticles promote acute-on-chronic alcoholic neutrophilia and hepatotoxicity	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic-plus-single-binge ethanol: Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/6, Jnk1 –/– , Jnk2 –/– e Chop –/– female mice / 70 – 96 days old	**C57BL/6 mice:** - Dilation of the endoplasmic reticulum in the liver; - Increased expression of genes related to oxidative stress in the endoplasmic reticulum (Chop, Ero1a, Gadd34, Bip, Bim, Bak and Dr5). - Infiltration of hepatic neutrophils; - Marked regulation in the hepatic expression of genes associated with the inflammasome (Casp1, Nlrp3, and Il1b) - Presence of CYP2E1 protein microparticles in hepatocyte-derived mtDNA **Jnk1 -/- mice:** - Hepatic steatosis; **Jnk2 -/- mice:** -Reduction of ALT and AST; - Reduced hepatic expression of CHOP protein - Reduction in the expression of hepatic mRNAs related to oxidative	This study investigated patterns of alcohol consumption in humans and animals to understand the burden of extracellular vesicles - key players in inflammation and neutrophil infiltration in liver injury. The findings show that the protocol applied to experimental animals induces liver damage through inflammation due to the accumulation of neutrophils and an increase in microparticle mtDNA, caused by increased oxidative stress in the endoplasmic reticulum.

						stress (Bim , Dr5 , Chop , Ero1a and Gadd34) - Reduction in genes associated with the inflammasome; **Chop -/- mice:** -Reduction of ALT and AST - Marked increase in steatosis compared to WT; - Reduction in genes associated with the inflammasome;	
48	The Cannabinoid Receptor 2 Protects Against Alcoholic Liver Disease Via a Macrophage Autophagy-Dependent Pathway	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic-plus-single-binge ethanol: Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/6N, Myeloid cell-specific CB2 or ATG5 mice / age not informed	**C57BL/6N mice:** - Positive F4/80 reduction - Slight increase in the number of neutrophils recruited - Increase in hepatic triglycerides - Increased hepatic steatosis **CB2 Mye -/- mice:** - Intense increase in the expression of pro-inflammatory genes (CCL3, IL-6, IL-1β, IL-1α and TNF-α); - Increase in the recruitment of neutrophils; - Accumulation of hepatic lipids and triglycerides; - Increased autophagy regulatory genes (LC3 and SQSTM1) **ATG5 Mye -/- mice:** - Increased hepatic steatosis - Increased hepatic steatosis - Increased mRNA	This study investigated the mechanisms of CB2 receptor action in liver-resident macrophages and whether its effects are dependent on autophagic activation. These mechanisms were explored by inducing hepatic alterations in different murine strains by chronic-plus-binge ethanol feeding. Binge drinking induced liver damage and steatosis, as well as inflammation. This damage was attenuated by activation of the CB2 receptor in Kupffler cells, and its protective effect is due to an autophagy-dependent pathway.

						expression of CCL4, IL-1α, CCL3 and IL-6	
49	Cytochrome P4502E1, oxidative stress, JNK, and autophagy in acute alcohol-induced fatty liver	Binge-like ethanol	The first dose was administered intraperitoneally at 0.93g/kg. Three doses, administered intragastrically, at a dose of 1.25g/kg	Accumalative dosage: Four doses at 3-minute intervals.	C57BL/6J, SV/129, jnk1−/− and jnk2−/− male mice / 56 – 70 days old	**C57BL/6J mice:** - Increased expression of CYP2E1 - Increase in triglycerides - Increase in fat vesicles; - Increased activation of JNK - Increase in oxidative stress markers (4-HNE and TBARS) - Reduced LC3-II/LC3-I, associated with autophagy - Increased expression of genes that synthesize fat (SREBP) **JNK1 KO mice:** - Increase in hepatic triglycerides **JNK2 KO mice:** - Increase in hepatic triglycerides	The authors of this article sought to understand the burden of CYP2E1 and which mechanisms associated with this protein are involved in the development of hepatic steatosis caused by binge drinking. The present findings demonstrate that binge drinking can induce hepatic steatosis through increased CYP2E1 expression, oxidative stress, increased fat synthesis and impaired autophagy,
50	Ethyl pyruvate ameliorates acute alcohol-induced liver injury and inflammation in mice	Binge-like ethanol	5g/kg, intragastric Route	Three intragastric administrations, 12 hours apart.	C57BL/6 female mice / age not informed	- Increase in ALT; - Increase in MDA; -Increase in inflammatory markers	This study aims to understand whether Ringer's Ethylpyruvate can reverse the late effects of binge drinking. The findings show that the methodology used induces liver damage through inflammation and oxidative stress.
51	Brain Injury and Recovery Following Binge Ethanol: Evidence from In Vivo Magnetic Resonance Spectroscopy	Binge-like ethanol	Loading dose: 5 g/kg, intragastrically Binge-like ethanol: 3 g/kg, intragastrically	Loading dose of ethanol (5 g/kg) intragastrically, followed by 3 g/kg every 8 hours for 4 days..	Wistar male rats / age not informed	- No liver changes 7 days after binge drinking	This study sought to understand the neurological damage caused by binge drinking in a murine model using magnetic resonance imaging and spectroscopy; however, as a complement to this knowledge, liver patterns were evaluated. In this study, no hepatic alterations were

							demonstrated 7 days after exposure to ethanol.
52	Anti-inflammatory function of ginsenoside Rg1 on alcoholic hepatitis through glucocorticoid receptor-related nuclear factor-kappa B pathway	Binge-like ethanol	6g/kg, intragastric Route	Intragastric administration of ethanol once daily for 9 days.	C57BL/6 male mice/ age not informed	- Increased ALT, AST, triglycerides, cholesterol and lactate dehydrogenase. - Mitochondria partially disappeared in the damaged hepatocytes; - Increase in inflammatory cytokines (TNF-α, IL-1β and IL-6);	The authors of this study aimed to understand the mechanisms of Rg1 in liver injury induced by binge drinking. The protocol applied in a murine model induced liver injury and tissue inflammation. These alterations were subsequently attenuated by Rg1, possibly linked to its hepatoprotective and anti-inflammatory activity
53	Inhibition of Type I Natural Killer T Cells by Retinoids or Following Sulfatide-Mediated Activation of Type II Natural Killer T Cells Attenuates Alcoholic Liver Disease in Mice	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic-plus-single-binge ethanol: Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL / 6J (B6) and Jα18 −/− male mice / 56-70 days old	**C57BL / 6J (B6) mice**: - Increased ALT and AST; - Accumulation of NKT type 1 - Increased secretion of IFNy - Increase in pro-inflammatory genes (IL-1β, IL-6 e OPN) **Jα18 -/- mice:** - Reduction in ALT; - Increase in pro-inflammatory genes	This study sought to elucidate how the differential activation of natural killer T (NKT) cells influences the inflammatory picture produced during alcoholic liver disease because of binge drinking. The chronic-plus-single-binge ethanol feeding protocol induced liver damage and inflammation mediated by NKT activation and its inflammatory cascade.
54	Acute Ethanol Causes Hepatic Mitochondrial Depolarization in Mice: Role of Ethanol Metabolism	Binge-like ethanol	1 - 6g/kg, intragastric rout	Single intragastric administration of ethanol	C57BL/6, Cyp2E1–null male mice/ 53 – 63 days old	**C57BL/6 mice:** -Mitochondrial depolarization increased progressively as ethanol doses were increased; - Polarization of mitochondria in ADH-positive and ADH-negative animals; - Increase in acetaldehyde adducts; - Increase in oxidative stress markers - Increase in ALT and decrease in ATP; **Cyp2E1–null mice:** - Reduced mitochondrial dyspolarization	This study determined how binge drinking impairs hepatic mitochondrial polarization and what the consequences of drug metabolism are on steatosis. The authors demonstrate that alcohol consumption produces hepatic mitochondrial depolarization, which may be an important factor in susceptibility to liver damage, through ATP impairment and hypoxia. The alcohol protocol also produced liver damage and steatosis associated with alcohol metabolism.

55	Alcohol increases tumor necrosis factor α and decreases nuclear factor-κB to activate hepatic apoptosis in genetically obese mice	Binge-like ethanol	2,5 g/kg, intragastric route	Intragastric administration of ethanol once daily for 3 days..	C57BL/6J ob/ob and lean +/+ male mice / “Young” mice	**+/+ mice:** - No liver damage; - Reduced GSH peroxidase and GSH S -transferase activity **Ob/ob mice:** - Microvacuolar steatosis; - Necrotic hepatocytes with inflammatory infiltrate; - Increased ALT; - Increased caspase-3; - Increased TNF-α	This study aimed to investigate and compare the hepatic effects of binge ethanol in lean and obese animals. The protocol applied to lean animals induced apoptosis and little change in oxidative balance. In contrast, obese mice exhibited more pronounced apoptosis, although there was no change in oxidative stress, which may be related to an adaptive process in the animal organism.
56	Increased hepatic receptor interacting protein kinase 3 expression due to impaired proteasomal functions contributes to alcohol-induced steatosis and liver injury	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic-plus-single-binge ethanol: Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57Bl/6J, RIP3 KO and RPT2 KO male mice / age not informed	**C57BI/6 mice:** - Increase in hepatic RIP3; - Increase in ALT and triglycerides; - Increased expression of CYP2E1; - Increased inflammatory genes (IL-6, MCP-1 and TNF-α) - Infiltration of neutrophils - Reduction in RIP1 protein levels **RIP3 KO mice:** - Decrease in ALT and inhibition of the increase in triglycerides;; - Increased expression of CYP2E1; - No expression of hepatic RIP3; - Infiltration of neutrophils **RPT2 KO mice:** - Increase in RIP1 and RIP3 protein; - Decline in hepatic PSMC1 protein;	This study investigated how chronic alcohol consumption and binge eating affect hepatic receptor-interacting protein (RIP) 3 kinase. The results show that alcohol consumption can induce hepatic steatosis, inflammation and necroptosis, associated with the activity and accumulation of hepatic RIP3 and RIP1. In addition, it was observed that RIP3 KO animals were more resistant to liver damage and steatosis.
57	DEP domain-containing mTOR-interacting protein suppresses	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol	Chronic-plus-single-binge ethanol: Chronic ethanol feeding for 10 days, ad libitum and orally, with a		- Accumulation of lipids inside the hepatocyte;	This study investigated the mechanisms involved in the development of alcohol-induced hepatic steatosis, supporting the

	lipogenesis and ameliorates hepatic steatosis and acute-on-chronic liver injury in alcoholic liver disease		diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/6 mice / 98 – 140 days old	- Increase in ALT and triglycerides; -Increased autophosphorylation of Mtor	hypothesis that rapamycin complex 1 may be linked to the development of liver injury, inflammation and steatosis. Evidence was found that binge drinking induces hepatic steatosis and obvious tissue inflammation, in which mTORC1 plays a key role in metabolic dysregulation, which contributes to alcoholic liver disease.
58	Invariant natural killer T cells contribute to chronic-plus-binge ethanol-mediated liver injury by promoting hepatic neutrophil infiltration	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic-plus-single-binge ethanol: Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL / 6J, IL-4 −/− , IFN-γ −/− e CD1d −/− female mice / 70 – 84 days old	**C57BL / 6J mice:** - Increase in iNKT cells; - Reduction of L-selectin in iNKT cells; - Increase in ALT and AST; - Increased expression of pro-inflammatory genes (TNF-α, IL-1β and IL-6) - Increased expression of genes associated with fibrogenesis - Infiltration of neutrophils; **CD1d −/− and Jα18 −/− mice:** - Attenuated hepatic steatosis - Reduced fat accumulation - Reduction in pro-inflammatory genes - Decreased expression of genes associated with fibrogenesis - Reduced neutrophil infiltration;	The authors of this study evaluated the function of natural killer T cells in the process of inflammatory neutrophil infiltration in the liver of animals exposed to chronic-plus-single-binge ethanol feeding. The results of this study showed liver damage and inflammation, associated with neutrophil infiltration.
59	Increased Ethanol-Inducible Cytochrome P450-2E1 and Cytochrome P450 Isoforms in Exosomes of Alcohol-Exposed Rodents and Patients with	Binge-like ethanol	6 g/kg, intragastric route	Three intragastric administrations, 12 hours apart.	Fischer 344 WT female rats; Cyp2e1-null female mice / age no informed	**Fischer F344 mice:** - Accumulation of fat in hepatocytes; - Slight necrosis; - Increase in ALT and triglycerides; - Infiltration of neutrophils; - Increase in inflammatory cytokines	The authors of this study investigated the burden of CYP2E1 and other proteins in extracellular vesicles (EVs) in binge drinking of ethanol and their effects on oxidative damage in hepatocytes. This study demonstrated that experimental animals showed liver damage as a result of binge drinking. The mechanisms involved are due to

	Alcoholism Through Oxidative and Endoplasmic Reticulum Stress					- Increase in hepatic protein extracellular vesicles CYP2E1, CYP2A3, CYP1A/2 and CYP4B - Increase in reactive oxygen species **Cyp2e1-null mice:** - Increase in EV CYP2A5 - Elevation of CYP2A5 and CYP4B	an increase in CYP2E1 and its extracellular vesicles, causing an increase in oxidative stress. They also induced inflammation.
60	Mitochondrial Double-Stranded RNA in Exosome Promotes Interleukin-17 Production Through Toll-Like Receptor 3 in Alcohol-associated Liver Injury	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 4 g kg, intragastric route	Chronic-plus-single-binge ethanol: Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/6 and TLR3 KO male mice / 56 – 70 days old	**C57BL/6 mice:** - Increase in ALT and AST; - Increase in the production of IL-17A by Kupffer cells; **TLR3 KO mice:** - Reduction of IL-17A-producing lymphocytes in γδ T cells; - Kuppfler cells with decreased expression of Il1b, Il23a and Ccl20 mRNA; - Reduced ALT and triglycerides; - Reduced expression of mRNAs involved in neutrophil and macrophage recruitment: Cxcl1 and Cxcr2	The authors of this study investigated the burden of hepatic mitochondrial double-stranded RNA on the progression of alcoholic liver disease, induced by binge drinking. The protocol applied induced injury and inflammation in the liver, associated with massive IL-1β production from Kupffer cells, mediated by TLR3 activation. These findings make these mechanisms an important therapeutic target.
61	Luteolin Alleviates Alcoholic Liver Disease Induced by Chronic and Binge Ethanol Feeding in Mice	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally.	Chronic-plus-single-binge ethanol: Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/ 6 male mice. / 42 days old	- Increased ALT and AST - Increased triglycerides and LDL cholesterol; - Presence of lipids in hepatocytes - Increased expression of genes that synthesize fat	The authors of this study sought to understand the actions of Luteolin on liver damage and steatosis induced by binge drinking. The findings show that alcohol consumption induces steatosis, inflammation and liver damage. These changes were significantly reversed by

			Binge ethanol: 4 g kg, intragastric route			(Srebp1c, Fasn , Ac and Scd1) - Increase in inflammatory markers (IL-1β and IL-6)	the use of Luteolin, a flavonoid that could be a possible therapeutic target in alcoholic liver disease.
62	Increased liver-specific proteins in circulating extracellular vesicles as potential biomarkers for drug- and alcohol-induced liver injury	Binge-like ethanol	6 g/kg, intragastric rout	Two intragastric administrations, 12 hours apart.	BALB/c male mice/ 42 days old	- Mild or moderate increase in hepatic steatosis; - Increased albumin, haptoglobin and fibrinogen in extracellular vesicles;	The authors of this study aimed to understand the role of circulating extracellular vesicles as a possible marker of liver injury, induced by binge drinking and paracetamol use. The results obtained show that binge drinking induced liver damage, which was observed by an increase in total and liver-specific proteins.
63	Activation of toll-like receptor 3 attenuates alcoholic liver injury by stimulating Kupffer cells and stellate cells to produce interleukin-10 in mice	Binge-like ethanol	C57BL /6 and TLR3 -/-: 4g/kg, intragastric route IL-10 -/-: 3g/kg, intragastric route	Intragastric administration of ethanol once daily for 2 weeks.	C57BL/6, TLR3 -/- and IL-10 -/- male mice /	**C57BL/6 mice:** - Increased ALT, AST and triglycerides; - Increase in inflammatory cytokines (TNF-α, MCP-1 and IL-6); - Increased expression of genes that synthesize fat (SREBP1c and FAS); - Increased neutrophil infiltration; **TLR3 -/- mice:** - Increased ALT and AST;; - No change in IL-10 expression of HSCs **- IL-10 -/- mice:** - Increased expression of TNFa, MPC-1 and IL-6	The authors of this study investigated whether Toll-like receptor 3 activation by Kuppler cells and hepatic stellate cells has an effect on the progression and development of liver damage induced by binge drinking. The results show that alcohol treatment induced liver damage through liver inflammation and fat accumulation. These findings were attenuated by the activation of TLR3, which occurred through polyinosinic-polycytidylic acid.
64	β-Caryophyllene protects against alcoholic steatohepatitis by attenuating inflammation and metabolic dysregulation in mice	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	Chronic-plus-single-binge ethanol: Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/6J male mice / age not informed	- Increased ALT levels; - Presence of hepatocyte ballooning and microvesicular steatosis; - Development of neutrophilic infiltrates; - Pro-inflammatory phenotypic alteration `M1` of Kupffer cells	This study aimed to investigate the role of β-caryophyllene in liver injury induced by chronic plus ethanol feeding. This form of consumption induced hepatocyte damage, pro-inflammatory changes and steatosis. These conditions were attenuated with the use of β-caryophyllene.

						- Reduced and rounded macrophages with a less tree-like morphology; - Increase in pro-inflammatory lymphocyte markers;	
65	L-Serine Supplementation Attenuates Alcoholic Fatty Liver by Enhancing Homocysteine Metabolism in Mice and Rats	Binge-like ethanol	5 g/kg	Three intragastric administrations, 12 hours apart.	C57BL/6 male mice / age not informed	- Increased accumulation of lipids and triglycerides; - Increase in ALT; - Increased homocysteine - Increased expression of proteins that synthesize fats;	This study aimed to evaluate the role of L-serine in alcoholic hepatic steatosis induced by alcohol consumption or feeding. The authors showed that binge drinking induced liver damage and steatosis. These findings may be related to an increase in homocysteinemia, which may decrease oxidative stress in the endoplasmic reticulum and consequently increase the synthesis of fatty acids. These results were attenuated by treatment with L-serine.
66	Binge Alcohol-Induced Microvesicular Liver Steatosis and Injury are Associated with Down-Regulation of Hepatic Hdac 1, 7, 9, 10, 11 and Up-Regulation of Hdac 3	Binge-like ethanol	4,5 g/kg, intragastric route	Three intragastric administrations, 12 hours apart.	C57BL/6J male mice/ 56 days old	- Reduced hepatic histone deacetylase activity and increased hepatic histone acetylation; - Increase in ALT; - Increase in triglycerides and fat microdroplets in hepatocytes; - Positive regulation of the gene associated with hepatic FAS. - Negative regulation of the gene associated with β-oxidation (Cpt1a);	The present study evaluated how binge drinking influences the expression of HDACs and their burden in the development of liver alterations. The authors observed that binge drinking induced liver damage and steatosis. These results reinforce the link between the development of hepatic steatosis and alterations in Hdac expression, which end up affecting lipid synthesis and oxidation processes.
67	Mouse and Human Liver Contain Immunoglobulin A-Secreting Cells Originating From Peyer's Patches and Directed Against Intestinal Antigens	Chronic-plus-single-binge ethanol feeding	Chronic-plus-single-binge ethanol feeding: Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 4 g kg, intragastric route	Chronic-plus-single-binge ethanol: Chronic ethanol feeding for 10 days, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet. Followed by a single intragastric administration of ethanol.	C57BL/6-Ly5.1 mice / 70 – 112 days old	- Increase in IgA-secreting cells - Increase in serum IgA - IgA accumulation in liver tissue - Increase in ALT and AST; - Hepatic steatosis;	The aim of this study was to elucidate IgA production by liver B cells after binge drinking in mice and by human liver samples. The results obtained revealed that binge drinking in a murine model induced injury and steatohepatitis, as well as increasing the number of cells that secrete IgA in the liver. Treatment with FTY720 - which prevents the release of IgA-secreting cells from Peyer's patches -

							attenuated the changes caused by liver damage.
68	Ethanol hinging exacerbates sinusoidal endothelial and parenchymal injury elicited by acetaminophen	Binge-like ethanol + acetaminophen	Binge-like ethanol: 4 g/kg, intragastric route. Acetaminophen: 150 and 300 mg/kg, intragastric route	Total of 5 administrations, 12 hours apart or 3 weekly binges. 12 hours later, acetaminophen was administered.	C57Bl/6 male mice / 49 – 56 days old	This study also observed an increase in SPERBP-1 and FAS in animals exposed to binge ethanol.	This research aimed to evaluate the burden of binge drinking on liver sinusoidal endothelial cells, concomitant with the use of paracetamol.
69	Inflammation Is Independent of Steatosis in a Murine Model of Steatohepatitis	High-fat diet (HFD) + ethanol binge model	Binge ethanol: 5g/kg, intragastric route	HFD diet for 3 months, ad deliberum, orally. Followed by a single dose of intragastric ethanol	C57BL/6J and *Pparg Hep-/-* male mice / 56 – 84 days old	**C57BL/6J mice:** - Hepatic steatosis; - Infiltration of neutrophils - Increased AST and ALT; - Increase in Cxcl1 expression and protein; - Increased hepatic expression of Fsp27 **Pparg Hep-/- mice:** - Mild hepatic steatosis; - Low triglecerides levels - Increased AST and ALT; - Expressive neutrophil infiltration; - Intense increase in Cxcl1 expression; - Reduction in Fsp27a and Fsp27b mRNA levels	This study sought to understand the role of peroxisome proliferator regulation (PPARγ) in the development of steatosis and neutrophil infiltration in response to steatohepatitis induced by a high-fat diet and binge drinking. The results revealed that the protocol used caused inflammation, injury and fat accumulation in the liver of wild-type animals. However, animals deficient in Pparg Hep-/- exhibited only a slight accumulation of fat, but with an intense infiltration of neutrophils. This suggests that the effects may be mediated by different mechanisms.
70	Oroxylin a promotes PGC-1α/Mfn2 signaling to attenuate hepatocyte pyroptosis via blocking mitochondrial ROS in alcoholic liver disease	Chronic-plus-single-binge ethanol feeding	Lieber-DeCarli ethanol diet: 5% (v/v) ethanol, orally. Binge ethanol: 5 g kg, intragastric route	8 weeks, ad libitum and orally, with a Lieber-DeCarli liquid ethanol diet, plus multiple compusions, with intragastric administration - twice a week during chronic feeding	ICR male mice / 48 – 56 days old	- Accumulation of lipid droplets; - Increase in ALT; - Increase in triglycerides; - Activation of the NLRP inflammasome; - Increase in ROS;	This study investigated the role of Oroxylin in hepatocyte pyroptosis in alcoholic liver disease induced by chronic plus binge ethanol feeding. Alcohol treatment caused significant liver damage, manifested by a disorganized lobular structure, infiltrative inflammation, fat accumulation in the liver and an increase in hepatocyte size.
71	Apoptosis of enterocytes and nitration of junctional complex proteins promote alcohol-	Binge-like ethanol	6g/kg, by intragastric gavage	Three intragastric administrations, 12 hours apart.	Fischer 344 female mice / 42 – 56 days old	- Hepatic steatosis; - Increase in triglycerides; - Increased ALT; - Increase in CYP2E1, iNOS, nitrated proteins and TLR-4;	This study aimed to explore the mechanisms of intestinal and liver damage induced by binge drinking. The results show that binge drinking induced liver damage, steatosis, and

	induced gut leakiness and liver injury	- Increased TNF-α and MCP-1	inflammation. In addition to marked changes in enteric physiology.

Legend: ALT= alanine transferase; AST= aspartate transferase; CCR2= C-C chemokine receptor type 2; MCP1= monocyte chemoattractant protein-1; IL- 6= interleukin-6; GSH= glutathione; 4-HNE: 4-hydroxy-2-nonenal; SREBP-1= sterol regulatory element binding protein 1; FAS= fatty acid synthase; LXRα= liver X receptor; ACC= acetyl-CoA carboxylase; SCD-1= stearoyl-CoA desaturase 1; PPARα = peroxisome proliferator-activated receptor alpha; miR-22 = microRNA-22; GFP-LC3 = green fluorescent protein-LC3; TBEF = EB transcription factor; mTOR = target of rapamycin; mTORC1 = target of rapamycin complex 1; REG3G = regenerating islet-derived gamma protein 3; miR-148a = microRNA 148a; Txnip = thioredoxin-interacting protein; Fox01 = forkhead box protein O1; NLRP3 = NLR family pyrin domain containing 3; mtDNA = mitochondrial DNA; MDA = malondialdehyde; miR-223 = microRNA 223; p47phox = neutrophil cytosolic factor 1; GSDMD = Gasdermin D; IL-17 = Interleukin-17; IL-18 = Interleukin-18; Casp11 = caspase 11; CYPE21= Cytochrome P450 2E1; pAMPK= ACTIVATED AMP-PROTEIN KINASE EXPRESSION; HFD= high fat diet; CXCL1= chemokine ligand 1 with C-X-C motif; Foxo3a = forkhead box O 3a transcription factor; Acox1 = acyl-CoA oxidase 1; Ac-H3-LYS9;= acetyl-histone H3 (Lys9); mtSSB = mitochondrial single-stranded DNA-binding protein; TBARS = thiobarbituric acid reactive species; NOS= nitric oxide synthase; iNOS, inducible nitric oxide synthase; NKT= natural killer T; JNK= c-Jun N-terminal kinase; LC3-II= LC3-phosphatidylethanolamine conjugate; TLR2= toll like receptor 2; TLR4= toll like receptor 4; TLR4P=toll like receptor 9; TNF-α=tumor necrosis factor alpha; FGF21=fibroblast growth factor 21; SCD1=stearoyl-CoA desaturase 1; TH17= T helper cells 17; FASN = fatty acid synthase; ACC-1 = acetyl-CoA carboxylase 1; HCFD = high cholesterol and saturated fat diet; MPO = myeloperoxidase; SPP1 = secreted phosphoprotein 1; MCP1 = monocyte chemoattractant protein-1; MIP2= macrophage inflammatory protein-2; SREBF1= sterol regulatory element binding transcription factor 1; ACLY= ATP citrate lyase; Nrf2= nuclear factor E2; NF-κB= nuclear factor kappa B; α-SMA= smooth muscle alpha actin; CHOP= protein homologous to transcription factor C/EBP; ERO1A= endoplasmic reticulum oxidoreductase 1 alpha; GADD34= regulatory subunit 15A of protein phosphatase 1; SCD1= stearoyl-CoA desaturase; HSC= hepatic stellate cells; AMPK= adenosine 5' monophosphate-activated protein kinase; LX-2= human hepatic stellate cell line; bip= immunoglobulin binding protein; BIM= cell death mediator that interacts with Bcl-2; BAK= BCL2 antagonist/killer 1; DR5= death receptor 5; IL-1β= interleukin-1 beta; IL-1α= interleukin-1 alpha; CCL3= chemokine ligand with C-C motif 3; SQSTM1= sequestosome-1; WT= wild type; KO= knockout; JNK= c-Jun-N-terminal kinase; IFN-γ= interferon gamma; OPN= osteopontin; ADH= alcohol dehydrogenase; RIP3= receptor-interacting protein kinase 3; RIP1= receptor-interacting protein kinase 1; iNKT= invariant natural killer T; CYP2A3= cytochrome P450, family 2, subfamily a, polypeptide 3; CYP4B= cytochrome P450, family 4, subfamily B, polypeptide 1; LDL = low-density lipoprotein; MPC-1 = mitochondrial pyruvate transporter 1; Carnitine O-palmitoyltransferase 1; igA = immunoglobulin A; Fsp2 = fat-specific protein 27; EVs= extracellular vesicles.

Among the experimental documents evaluated, 8 proposed a relationship between binge ethanol administration protocol and the stages of alcoholic liver disease (ALD). Of these, 2 authors evaluated different protocols within the same modality (Figure 10). The results obtained allowed us to evaluate that protocols involving only treatment with alcohol, without any secondary insult, induce only early alcoholic liver disturbances in animals - single administration; intermittent and episodic. On the other hand, studies that associated chronic feeding with alcohol for 10 days, followed by a binge episode, resulted in a picture of early steatohepatitis - Chronic ethanol (CE) 10 days plus a single binge. When the ethanol feeding protocol was extended to 8 weeks, followed by one or multiple binge episodes, the development of moderate steatohepatitis was observed in the experimental animals - CE 8 weeks plus single binge. Curiously, a model that involved hypercaloric feeding for 3 days, followed by a single administration of ethanol, also reproduced only moderate steatohepatitis – High fat diet (HFD) 3 days plus a single binge. However, studies that applied a caloric feeding regime for 3 months, along with a binge episode of ethanol, resulted in severe steatohepatitis - HFD 3 months plus a single binge. Furthermore, a protocol that administered hypercaloric and saturated feeding for 2 weeks, followed by 8 weeks of alcohol feeding and a single administration of alcohol, reproduced severe chronic steatohepatitis – High cholesterol, and saturated fat diet (HCFD) 2 weeks, plus alcoholic diet for 8 weeks plus a weekly binge.

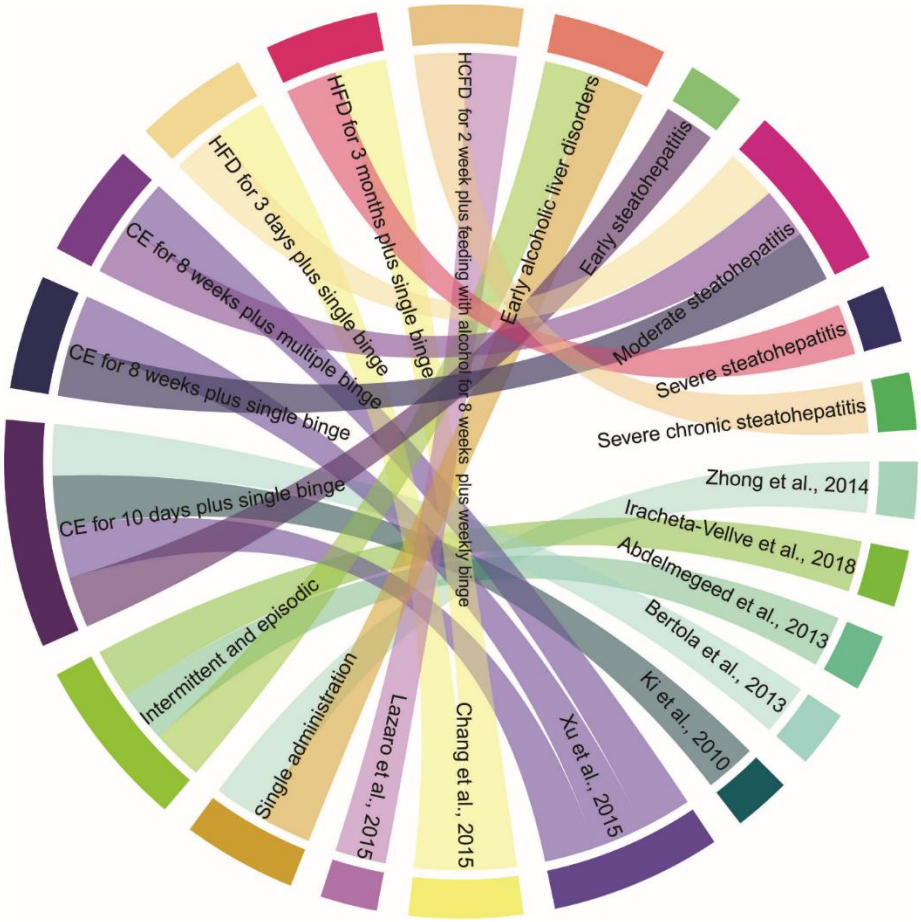

Figure 10. Circular migratory flow. Figure representing the types of ethanol binge, the corresponding administration protocol, and the respective stages of Alcoholic Liver Disease (ALD), according to the author's classification.
Chronic ethanol: CE; HFD: High-fat diet; HCFD: High cholesterol, and saturated fat diet.

The results presented demonstrate that binge-like ethanol can produce a variety of liver damage. The most explored mechanisms involved include hepatic steatosis, tissue inflammation, oxidative stress, hepatocyte apoptosis, mitochondrial dysfunction, activation of hepatic stellate cells, and dysfunction in lipid metabolism. It should be noted that these results may vary in intensity and presentation according to the protocol used.

Discussion

Here, we synthesize the results of the 100 most cited articles and contribute to the ongoing discussion on the pathogenesis of ALD, mapping the knowledge on alcohol

hepatotoxicity in different binge drinking protocols. Our findings revealed that Gao B stood out as the most prolific author, with 18 publications and 2,559 citations. The journal Hepatology emerged as the leading platform for disseminating these studies, with the terms "hepatic steatosis" and "alcohol" being the most frequent. The United States accounted for the majority of the scientific output. Furthermore, we observed that the severity and underlying mechanisms of liver injury vary according to the specific binge-like pattern, and the main factors contributing to the development of hepatic alterations include inflammatory processes, oxidative stress, and lipid metabolism dysregulation [62, 74, 35].

Binge ethanol consumption can induce several liver disorders that characterize ALD, such as hepatic steatosis, steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma, which these pathologies are responsible for the main causes of morbidity and mortality associated with the liver system [128, 104]. Understanding the impact of consumption patterns on the progression of ALD may reveal new biological indicators for diagnosis or prognosis, as well as new therapeutic targets. Therefore, experimental models that simulate ALD are extremely necessary in scientific research [68]. The results obtained in this study show that all the experimental protocols analyzed used murine models (rats and mice), mainly C57BL/6 mice since this strain has well-defined properties and effectively reproduces the human clinical characteristics of ALD [50, 122, 129]. In addition, they are often used in studies that require genetic manipulation, such as transgenic or knockout mice [130].

Gender differences also play a fundamental role in the factors in binge ethanol consumption and its impact on liver function. In the experimental articles analyzed, it was observed that most of the studies were conducted on male animals and that none of these studies compared liver physiological differences after binge-like ethanol consumption. However, it is important to note that the literature shows that high BAC levels in female rats presented the enzyme alanine transaminase (ALT) and triglycerides levels higher than their counterparts following binge-like ethanol exposure, indicating that this sex is more susceptible to liver damage [131]. In addition, such females show elevated levels of cytochrome P4502E1 and altered signaling pathways, which may contribute to the development of ALD [132]. Although binge-type consumption causes disturbances in liver enzymes and increases levels of lipids, HDL-C, and total cholesterol in both sexes, the

intensity of the effects may vary [133]. Thus, although the changes occur in both females and males, the dysfunctions follow different pathways, highlighting that additional studies that address gender-specific therapies to mitigate the adverse effects of binge ethanol on liver health are required [131]. Overall, these findings reinforce the importance of gender factors in alcohol-related health research.

Currently, several experimental protocols for binge-like ethanol are employed. However, there is still no consensus on a standardized definition, due to the significant methodological diversity in studies investigating liver changes induced by binge drinking [50, 29, 74, 134]. Historically, research on these models has evolved from single or multiple administrations to compulsive administrations associated with secondary insults [74]. This progress has become necessary to more accurately represent the pathophysiological characteristics of human ALD.

One of the first methods used is known as 'binge-like ethanol', in which animals are exposed to excessive doses of ethanol, administered in one or several sessions [50, 113]. In this knowledge mapping, we found that, despite sharing the same nomenclature, a wide variety of experimental protocols use different administration regimes. The lack of standardization of binge-type protocols related to human exposure definitions results in difficulties in obtaining uniform data on liver alterations induced by binge ethanol. In general, these administration regimes have been found to induce predominantly mild liver damage, representing the initial stages of ALD in humans, that is characterized by an increase in the activity of the ALT [50, 135, 81, 33, 49], liver inflammation [26, 38, 49, 70], changes in mitochondrial DNA [44, 92], hepatic steatosis [54, 93], oxidative imbalance [33, 55] and other mechanisms underlying the injury [30, 57, 60, 112].

It is noteworthy that among the protocols classified as binge-like ethanol, different results have been observed in the intensity of damage from single ethanol administration protocols *vs*. interval doses or consecutive doses. Protocols using acute ethanol ingestion generally result in mild liver damage. This type of administration can cause a temporary increase in liver enzymes, mild inflammation, and slight hepatic steatosis, which are signs of the early stages of ALD [50, 92, 117, 49]. However, the severity of such damage tends to be limited due to the lack of repeated exposure to ethanol. In contrast, intermittent and episodic

doses have been shown to induce more pronounced damage, demonstrating that the process of repetition induces the accumulation of oxidative stress and inflammation, exacerbating liver damage (Figure 11). In these scenarios, in addition to a more significant increase in liver enzymes, more intense inflammation can be observed, as well as a greater intensity of steatosis and signs of mitochondrial damage compared to a single administration [60, 32, 26, 118, 81, 106, 104, 55, 128]. These effects may be closer to the advanced stages of ALD, although they still represent an early stage of the disease [136].

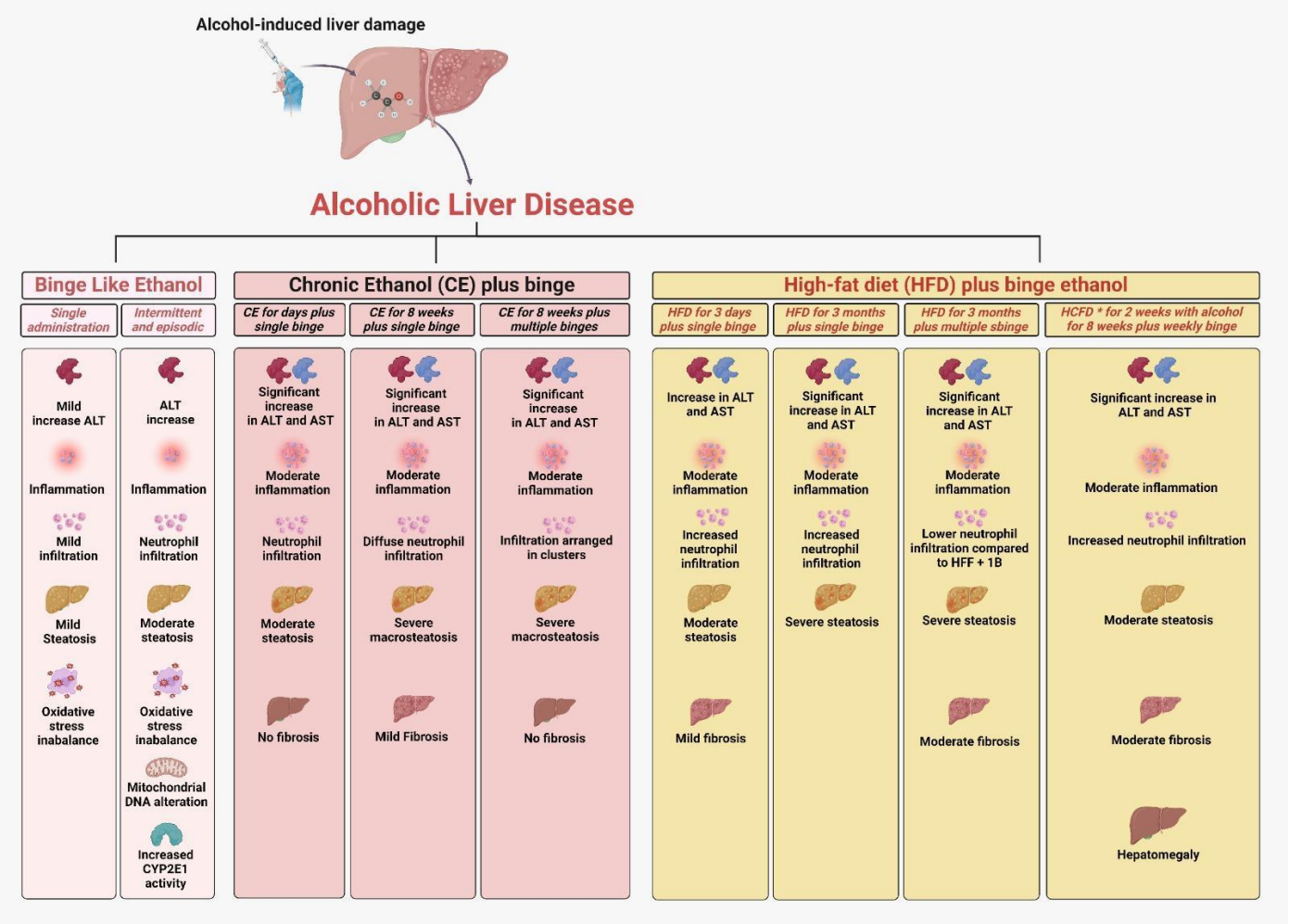

Figure 11. The most common liver changes observed in the evaluated binge ethanol experimental protocols. The figure shows a diagram comparing the effects of different patterns of alcohol consumption on the liver, using an animal model. The diagram illustrates how different alcohol administration regimes, including acute consumption (binge drinking), chronic consumption, and chronic consumption associated with a high-fat diet, affect the development of alcoholic liver disease.

Chronic ethanol: CE; HFD: High-fat diet; HCFD: High cholesterol, and saturated fat diet.

It is therefore essential to investigate the variability in liver response between the different administration regimens, which would benefit the scientific field in choosing the most suitable "binge-like ethanol" protocol, depending on the objective of the study. This differentiation also underlines the need to standardize protocols in order to allow for more accurate comparisons between different studies.

Given the widespread use of the “binge-like” ethanol consumption model, new ingestion methods have recently been investigated. In the last decade, several experimental models have been developed in which different approaches combine the use of food or chemical substances with binge-like ethanol consumption [137, 9]. One of these new models, known as the “mouse model of chronic and binge ethanol feeding (the NIAAA model)”, involves chronic alcohol feeding for short or long periods (using the Lieber-DeCarli diet), followed by compulsive alcohol consumption in one or several doses. This protocol was developed to induce more significant liver damage since the dissociation of the two components generates only mild steatosis, a small increase in the serum enzyme (ALT), and little or no inflammation [29]. However, in the combined protocol an increase in serum transaminase enzymes (ALT and aspartate aminotransferase-AST), along with marked steatosis, neutrophil infiltration, inflammation, and liver fibrosis [37, 29, 96, 30]. Even in this consumption model, distinct hepatic alterations were also observed. Short-term administration, that is, chronic alcohol feeding for 10 days followed by a binge-like episode (E10+1B), induces a less advanced model of alcoholic steatohepatitis (ASH) when compared to long-term models, such as 8 weeks of Lieber-DeCarli feeding followed by one or multiple binge episodes (E8W+1B and E8W+mB, respectively). Although the findings from the long-term administration protocols are comparable, the observed alterations can be explained by the nature of the injury, which E8W+1B represents an acute-on-chronic liver injury, while E8W+mB reflects a combination of chronic liver injury with acute-on-chronic liver injury [64].

In this context, Chang et al. (2015) and Lazaro et al. (2015) [51, 78] propose an approach based on a high-fat diet (both short- and long-term) combined with binge drinking.

These protocols aim to investigate the harmful effects on the liver resulting from the interaction between binge drinking and obesity. It was observed that the application of this method led to liver damage, severe steatosis, intense inflammation, and fibrosis [137, 51, 71], in addition to increased expression of lipogenic and inflammatory genes, accompanied by the downregulation of critical genes for metabolism and antioxidant status [51, 78]. Lazaro and collaborators (2015) [78] associated weekly binge with HFD and found mononuclear cell infiltration, and pericellular and perisinusoidal fibrosis, characterizing a severe form of ASH. Furthermore, this experimental protocol showed clinical features of alcoholic hepatitis, such as splenomegaly, hypoalbuminemia, and bilirubinemia.

Carmiel-Haggai et al. (2003) [85] used animals genetically predisposed to obesity (fa/fa) to examine the pattern of binge-like ethanol and found an increase in liver damage and programmed liver cell death in obese animals when compared to lean mice. These results suggest that alcohol consumption plays a key role in the development of ALD in obese individuals, indicating that this group faces a higher risk of developing more severe liver damage [10, 115]. In addition, the authors highlight the importance of protocols that explore the histological and molecular characteristics of secondary insults, observed in compulsive drinkers, with liver damage resulting from binge drinking [71, 138, 62].

In addition to the introduction of new procedures, new targets for action were also observed. When analyzing the set of keywords, we observed that terms related to the “gut” were used frequently in recently published documents. This is due to the growing interest in recent years in exploring the strong link between liver damage and intestinal alterations [77, 138, 140]. The results obtained indicate that binge-like ethanol facilitates the translocation of bacterial components from the intestine to the liver, resulting in endotoxemia, an inflammatory response, and subsequent liver injury [77, 69, 86, 49]. These findings suggest that investigating the gut-liver axis could open new avenues for developing therapies focused on modulating the intestinal microbiota and reducing systemic inflammation, to treat liver diseases. In line with these findings, our analysis revealed a strong association between the term 'hepatic' and the words 'microbiota' and 'inflammation,' further supporting this line of reasoning.

If we compare these findings with current research from 2024, we can see that this topic has been strengthened and expanded. Recent studies reinforce the complex interaction of the gut-liver axis, highlighting the role of the gut microbiome in modulating the inflammatory and oxidative processes triggered by binge ethanol consumption [141, 142, 143]. In addition, there has been progress in the investigation of dietary supplementation strategies, such as the use of probiotics and bioactive compounds, to mitigate liver damage and restore microbiological balance [144, 145, 146].

This evolution demonstrates that, while previously the focus was on identifying the isolated impacts of alcohol on the gut and liver, recent research is aimed at integrative therapeutic interventions. In addition, advances in microbiome analysis technologies and specific biomarkers have enabled a more detailed understanding of the underlying mechanisms.

The financial support of institutions such as the National Institutes of Health (NIH) has been fundamental in driving many advances in scientific knowledge, especially in the area of health. This US government body plays a crucial role in conducting research and applying this knowledge to improve the quality of life of the population [141].

Composed of 27 institutes and centers, the NIH directs resources to a wide variety of research, from fundamental studies to the development of innovative technologies and programs. These investments translate into significant research, which may explain the large number of scientific articles published in the United States since the NIH focuses most of its medical research resources on the American population. If we look at the figures, we can see that around 60% of the scientific articles selected are funded by the NIH, highlighting its great importance in advancing biomedical knowledge [141]. However, although it is a North American institution, the NIH also supports research in other countries, which boosts global science and facilitates international collaborations. In addition, the NIAAA, which pertains to the NIH, plays a key role in leading and funding studies on the effects of alcohol, intending to transform knowledge into measures to improve the diagnosis, prevention, and treatment of the population affected by alcohol consumption. These entities are key to boosting scientific research and tackling public health challenges at a global level.

These incentives can be seen in the increase in the production and dissemination of scientific knowledge. For example, the researcher Bin Gao, responsible for the largest number of contributions in the selected articles, is also a senior researcher at the NIAAA Liver Disease Laboratory. This researcher has become a reference on the subject, presenting a significant number of links in his articles, which demonstrates the extent of his contribution to research into liver damage caused by binge drinking. Another major contributor to the area explored in this work is Adeline Bertola, responsible for the document with the highest number of citations among the articles selected. This merit is due to her first authorship of the most cited article on the subject, published in Nature Protocol, which describes the chronic ethanol feeding methodology, also known as the Gao-binge model, and also identified as the “NIAAA model” that was used in more than 20% of the experimental articles analyzed.

It is relevant to note that the three journals with the highest number of publications were “Hepatology”, “Journal of Hepatology”, and “Gastroenterology”, highlighting that a significant portion of scientific production is focused on hepatic issues. This suggests a strong emphasis on research into liver diseases and potential associated treatments, reflecting the growing importance of this field in the current medical landscape. Following these, the journals "Alcohol Research and Health," "Free Radical Biology and Medicine," and "Gut" are also relevant to the analysis, as the evaluation of these journals' scopes demonstrates an integrated approach to studying liver diseases. The presence of these journals in the bibliometric review underscores the importance of exploring the impact of binge drinking from a multidisciplinary perspective. These publications cover everything from the direct effects of alcohol on the liver to broader interactions within the gastrointestinal system and the involved molecular processes.

The findings identified in this study highlight not only an increase in the volume of publications but also a broadening of the topics covered, which involve pathophysiological mechanisms, clinical impact, and potential intervention strategies. Despite significant progress in understanding the mechanisms of damage involved in liver binge drinking, there are still gaps, especially concerning longitudinal studies and innovative therapeutic approaches. Therefore, future research should investigate consistent and multidisciplinary

methodologies to deepen the understanding of this complex connection and assist in the formulation of effective public health policies.

Finally, a bibliometric analysis consists of a quantitative evaluation of data, enabling the measurement of scientific production on a specific topic and the identification of the main research trends. However, it is important to emphasize that this study, due to its quantitative nature, does not allow for an in-depth critical analysis of the documents, being limited to providing statistical indicators and a mapping of knowledge. Consequently, although it is a useful tool for exploring the topic, bibliometrics should not be considered a guide for clinical practice.

Conclusions

The synthesis of the results of the 100 most cited articles on alcohol hepatotoxicity and alcohol hepatic disease offers significant insights into understanding the underlying mechanisms and the diversity of liver lesions associated with its excessive consumption. The variation in lesion severity and pathophysiology identified in different models of binge drinking highlights the complexity of the pathogenesis of alcohol hepatic disease and the need for individualized approaches to diagnosis and treatment. The convergence of studies on animal models serves to highlight the relevance of these experimental systems in replicating the clinical characteristics of human alcohol hepatic disease. Furthermore, the identification of new experimental models, such as the "High Fat Diet + Binge Drinking" protocol, and the exploration of the role of the intestinal microbiota in the progression of alcohol hepatic disease provides new research perspectives and possible therapeutic targets. The funding provided by government agencies, such as the NIH, plays a pivotal role in advancing these studies, underscoring the significance of international funding and collaboration in comprehending and addressing the complexities associated with alcohol consumption and its hepatic consequences.

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Author contributions

All authors contribute to the data analyses of the study. Design of the work: R.R.L, L.M.P.F and T.P.T.M. Acquisition of data: T.P.T.M and, P.I.C.S. Analysis of data: T.P.T.M, L.V.P.S, B.C.C. Interpretation of data: T.P.T.M, M.V.O.R, L.V.P.S and B.C.C. Drafting of the manuscript: T.P.T.M, L.C.F.F, J.J.S.F, E.S.O and E.A.F.J. Critical revision of the manuscript: C.S.F.M, R.R.L and L.M.P.F. All authors revised and approved the final version of the manuscript.

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Conflict of interests

The authors declare that there is no conflict of interest.

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3.2 Estudo experimental

O estudo experimental foi fundamentado nos resultados do estudo bibliométrico, que analisou os 100 artigos mais citados sobre o padrão de consumo *BD* e suas consequências hepáticas. A análise indicou uma forte relação entre o consumo intermitente e episódico de EtOH e o desenvolvimento de alterações hepáticas significativas, como esteatose, inflamação, estresse oxidativo e fibrose, frequentemente mediadas por disfunções metabólicas e moleculares no fígado. Apesar da literatura disponível, permanecem questionamentos importantes quanto à compreensão dos efeitos a curto e longo prazo do consumo de EtOH em padrão BD, especialmente quando iniciado na adolescência e mantido até a fase adulta. Diante dessa lacuna, o estudo a seguir foi desenhado com objetivo principal de investigar as alterações hepáticas resultantes do consumo intermitente e episódico de EtOH da adolescência à fase adulta, bem como avaliar se essas alterações persistem após um período prolongado de abstinência.

O documento intitulado "ADOLESCENT BINGE-LIKE ETHANOL CONSUMPTION INDUCES SHORT- AND LONG-LASTING HEPATIC OXIDATIVE IMBALANCE AND LIVER DYSFUNCTION WITH CHANGES IN LIPIDIC METABOLISM" será submetido no periódico "Free Radical Biology and Medicine" que possui Fator de Impacto de 7.1 e com *Score* de Citação de 14.

Adolescent binge-like ethanol consumption induces short- and long-lasting hepatic oxidative imbalance and liver dysfunction with changes in lipidic metabolism

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Abstract

Excessive alcohol consumption, especially among adolescents, contributes to social, economic and health problems globally. Among age groups, adolescents stand out for consuming a greater amount of alcohol per occasion than adults. This practice is known as binge drinking (BD), characterized by the intermittent and episodic consumption of alcohol that raises the blood concentration to 0.08 g/dL or more. The BD pattern is related to a homeostatic imbalance in organs and tissues, especially in the liver, which is responsible for metabolizing alcohol. This process results in the formation of reactive oxygen species (ROS) and acetaldehyde, a primary metabolite with high hepatotoxicity. Liver damage caused by binge drinking can progress from steatosis to steatohepatitis, accompanied by inflammation and necrosis. However, it is still not completely clear how different cycles of alcohol intoxication during adolescence can impact the liver, both in the short and long term, and what mechanisms are involved. In view of this problem, this study aims to evaluate the effects of repeated episodes of BD during adolescence and their biochemical and morphological repercussions on the liver. To this end, adolescent Wistar rats (n=62) were divided into groups and subjected to binge drinking protocol with three consecutive days of ethanol (3.0 g/kg/day; 20% w/v) intragastric gavage administration or water for 8 weeks/cycles. Then, serum and liver samples were collected for histopathological and biochemical analysis of lipid and oxidative profiles at 24 hours and at 14 days after administration to evaluate short- and long-term effects, respectively. Our results showed a significant increase in lipid peroxidation following acute binge alcohol consumption, which remained elevated even after a period of abstinence. A similar pattern was observed in catalase enzyme activity, which stayed elevated over time. In contrast, superoxide dismutase (SOD) activity decreased with alcohol consumption and remained reduced 14 days after the last administration. Additionally, hepatocyte injury markers (AST and ALT) levels remained elevated in both immediate evaluations and after the abstinence period, as well as lipid metabolism products. Histological analysis revealed the presence of grade 2 steatosis, along with necrosis and liver fibrosis in the alcohol-treated animals**.** These findings indicate that BD can induce significant liver damage during adolescence, which may persist into adulthood. Further studies are needed to elucidate the mechanisms involved in the hepatotoxicity associated with this pattern of alcohol consumption

Introduction

Alcohol consumption is a globally widespread practice, perpetuated in cultural traditions and facilitated by its widespread social acceptance (Hoffmann, 1996). Considered a psychoactive drug with a sedative-hypnotic effect, ethanol (EtOH) continues to be one of the most consumed substances in all age groups, especially among adolescents and young adults (McCambridge, John McAlaney & Richard Rowe, 2011; Costadi et al., 2015). Easy access and its frequent association with leisure and pleasure have contributed to a pattern of increasingly early and dangerous consumption, generating alarming implications for public health, social and economic issues (Heim et al., 2008; WHO, 2018).

Among the patterns of alcohol consumption, binge drinking has gained prominence. This type of use is characterized by the episodic and excessive intake of alcohol in a short space of time, followed by periods of abstinence. Popular among adolescents, this behavior is strongly associated with risks such as the development of chronic diseases, involvement in accidents and greater predisposition to risk behaviors (NIAAA, 2017; Blakemore et al., 2018; Chung et al., 2018). Although these problems are not exclusive to adolescents, this group is particularly vulnerable due to their lack of experience with the effects of ethanol and developmental physiological factors (Crews et al., 2007; Chung et al., 2018; Conegundes et al., 2018). In fact, epidemiological studies show that adolescents who start consuming alcohol before the age of 15 are significantly more likely to develop lifelong alcohol dependence and health problems during adulthood (NIAAA, 2017; Kuntsche et al., 2017).

The definition of binge drinking is essential in epidemiology to assess the risks associated with this pattern. As described by the NIAAA (2004), binge drinking is characterized by a blood alcohol concentration of 0.08 g/dL or more, reached with four doses for women and five for men in a two-hour period, followed by abstinence. Among adolescents, this risk is aggravated due to their smaller body size and lower metabolic efficiency, consequently they reach higher blood alcohol concentrations with fewer doses, increasing their vulnerability to health damage compared to adults (Blakemore, 2014; Donavan, 2009).

Given this, the liver is one of the organs most affected. As the main organ responsible for ethanol metabolism, it detoxifies the drug through oxidative reactions (Ceni, Mello & Galli, 2014). In social consumers, alcohol oxidation occurs mainly through the enzymes alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). This metabolic pathway is limited because it depends on the coenzyme nicotinamide adenine dinucleotide (NAD) and can become saturated. However, binge drinking activates additional pathways, such as the microsomal pathway, which uses cytochrome P450 E1 (CYP2E1) and requires adenosine triphosphate (ATP), resulting in the production of reactive oxygen species (Cederbaum et al., 2012). The catalase pathway, which oxidizes less than 2% of the alcohol consumed, is is considered toxic, as it generates hydrogen peroxide (Cederbaum et al, 2012; Hyun et al., 2021).

Excessive alcohol consumption, common in binge drinking episodes, overloads this liver function. This results in the accumulation of acetaldehyde and the generation of reactive oxygen species (ROS), which induce an imbalance of oxidative stress and damage to liver cells (Albano, 2006). This imbalance is associated with various liver diseases, including steatosis, alcoholic hepatitis and cirrhosis (Lieber, 2004). Even after short periods of abstinence, markers of liver damage and oxidative stress can persist, indicating incomplete tissue recovery (Pi et al., 2021). Therefore, the impact of alcohol on the liver is profound and potentially long-lasting, especially in contexts of binge drinking.

Although the literature is clear on the liver damage caused by chronic binge drinking (Yang et al., 2014; Li et al., 2017), it is not yet well understood how repeated episodes of binge drinking initiated during adolescence impact liver function in adulthood. Our group has shown that repeated cycles of ethanol intake in female rats during adolescence can result in liver damage (Fernandes et al., 2018). However, the mechanisms that explain these findings are not yet fully understood. Therefore, this study aims to investigate the liver changes associated with intermittent and episodic ethanol consumption in adulthood, as well as whether these changes persist after a long period of abstinence

Materials and Methods

Animals

Female Wistar rats (n = 62) were obtained from the Evandro Chagas Institute (IEC) vivarium and sent to the Luiz Carlos de Lima Silveira vivarium, located at the Center for Biological and Health Sciences (CCBS) of the State University of Pará (UEPA). The animals were housed in collective cages, with a maximum of four per cage, in order to avoid the stress caused by isolation. They were kept in an air-conditioned room with a 12-hour light/dark cycle and had access to food and water *ad libitum*. All procedures were approved by the Experimental Animal Ethics Committee of the State University of Pará (UEPA) under license number 16/2023 and followed the guidelines of the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (2011).

Experimental groups and treatment

In order to reproduce the pattern of ethanol consumption commonly observed in human adolescents (Del Ciampo and Del Ciampo, 2024), this experiment adopted a binge drinking (BD) protocol, in which the animals were given ethanol (3.0 g/kg/day, 20% w/v) or distilled water (H2O dest.) intragastrically, once a day, for 3 consecutive days, followed by 4 days of abstinence, totaling 7 days per cycle. Treatment began on the 35th postnatal day (PND), when the rodents were randomly reassigned to the control and EtOH groups (8/group) and exposed to intoxication from the 35th to the 86th PND, a period that corresponds to the transition from adolescence to adulthood in this animal model. Evaluations were carried out after 8 cycles of BD (35-86 DPN) and 8 cycles of BD followed by 14 days of abstinence, without any administration (see figure 1).

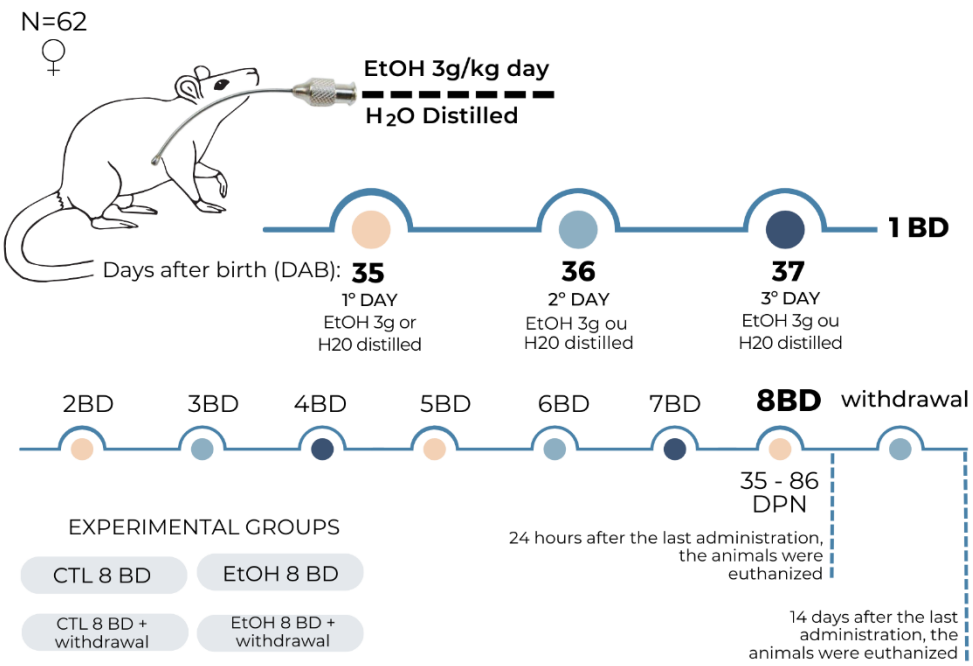

Figure 1: Schematic of the experimental treatment with EtOH. On the 35th day of life, the adolescent rats were randomly divided into two groups. The intoxication cycle with EtOH (3 g/kg/day) or distilled H_2O was defined as administration for three consecutive days. Following this protocol, the groups of animals were subjected to a total of 8 intoxication cycles, with immediate and delayed evaluation.

Biochemical tests

Samples

Twenty-four hours (short-term) and 14 days (long-term) after the end of the intoxication periods, the animals were anesthetized with isofluorane and then euthanized by cardiac puncture for biochemical evaluation. Blood was collected and stored in specific tubes for this purpose. At the same time, the liver was aseptically removed by total hepatectomy and cooled in liquid nitrogen. For analysis, the livers were thawed and weighed on an analytical scale, then added to a solution of PBS 1x (pH 7) in a ratio of 1:10. This mixture was homogenized and centrifuged to collect the supernatant, which was used in the biochemical analysis of liver lesions. The serum was obtained by centrifugation at 1400 ×g for 10 minutes and stored at -80°C until analysis

Analysis of biochemical parameters

The activities of serum triglycerides, very low-density lipoprotein (VLDL), alkaline phosphatase, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by the dichlorophenyldiazonium (DPD) method, UV optimized for AST and ALT, and by the specific method for determination of serum activity. The questions were performed using the CMD 600x1 device (Wiener Lab Group) and its respective kits.

Liver Oxidative Biochemistry

Determination of malondialdehyde (MDA): The method used is based on the change in color of plasma when reacting with 1% thiobarbituric acid in an acidic medium at 90-100°C. In Eppendorf-type microtubes (1.5 ml), 10 µL of BHT (2% ethanolic solution), 200 µL of 25% HCl, 200 µL of thiobarbituric acid solution (1% in 0.05N NaOH) and 200 µL of sample were added. A blank was prepared by replacing the sample with 0.9% saline solution. The tubes were incubated in a water bath (100°C) for 15 minutes, cooled on ice and then 618 µL of butanol were added, stirring until the pink color went to the top layer. After centrifugation at 12,000 rpm for 5 minutes, 200 µL of the supernatant (butanol phase) was removed and distributed in duplicate in a 96-well plate. The absorbance was measured at 532 nm in a spectrophotometer. The addition of BHT prevented the amplification of peroxidation during the assay (Brown & Kelly, 1996). The results were expressed as percentages of the control groups.

Catalase activity: The activity of the enzyme catalase was determined according to the method of Aebi (1984), which quantifies the decomposition of hydrogen peroxide at 240 nm for 20 seconds. The test was carried out on liver tissue and plasma from the experimental animals. A 10 mM solution of hydrogen peroxide (H_2O_2) in phosphate buffer pH 7.0 was prepared and titrated on the day of analysis. 2 mL of this solution was added to the cuvette, followed by 20 µL of the sample, and the drop in absorbance was measured.

Superoxide Dismutase (SOD) activity: SOD activity was measured spectrophotometrically at 550 nm, according to the method adapted from Flohé (1987). The enzyme catalyzes the

dismutation of superoxide into oxygen and hydrogen peroxide. The solutions prepared included: (a) 50 mM sodium phosphate buffer with 0.1 mM EDTA, Cytochrome C and Xanthine; (b) EDTA with Xanthine Oxidase. The test was carried out at 25°C, with the reaction medium added to the cuvette, followed by the sample. The blank procedure varied in the amount of solutions (a) and (b).

Liver Histopathology

Samples

For histological analysis of the liver, the animals were anesthetized intraperitoneally with a mixture of ketamine hydrochloride (90 mg/Kg) and xylazine hydrochloride (9 mg/Kg). The animals were then perfused transcardiacally - infusion of 0.9% saline solution to remove residual blood, followed by infusion of 2% formaldehyde to begin tissue fixation. After perfusion, total hepatectomy was performed and a tissue sample was taken from the medial portion of the left lobe, a region that includes perivenular areas - which are more susceptible to damage from hypoxia and alcohol toxicity (Xu et al., 1994). The samples were fixed in 10% formaldehyde for 48 hours and then in 10% buffered formalin. The tissues were then embedded in paraffin, sectioned into 4 μm-thick sections and stained with hematoxylin and eosin (HE).

Histopathological analysis

For histological analysis of the liver, the animals were anesthetized intraperitoneally with a mixture of ketamine hydrochloride (90 mg/kg) and xylazine hydrochloride (9 mg/kg). transcardiac perfusion was then performed, initially with 0.9% saline solution to remove residual blood, followed by infusion of 2% formaldehyde to begin tissue fixation. After perfusion, total hepatectomy was performed, with a sample taken from the medial portion of the left lobe of the liver. This region was selected because it included perivenular areas, known to be more susceptible to damage from hypoxia and alcohol toxicity (Xu et al., 1994). The samples were fixed in 10% formaldehyde for 48 hours and then transferred to 10% buffered formalin. The tissues were then processed for paraffin embedding, cut into 4 μm-thick sections and stained with hematoxylin and eosin (HE) for microscopic analysis.

Statistical analysis

The collected data was recorded, and statistical analysis was performed using GraphPad Prism software The one-way ANOVA test was applied,, considering the withdrawal periods, followed by Sidak's post hoc test for comparisons between groups. Statistically significant differences were considered for $p<0.05$.

Results

Repeated exposure to ethanol in a binge pattern induces long-lasting liver damage, detectable even after consumption has stopped.

The serum activities of liver damage parameters, assessed by alkaline phosphatase, AST and ALT levels, are shown in Figure 2. One-way ANOVA analysis (number of ethanol binge cycles) indicated a significant effect of ethanol treatment on alkaline phosphatase levels. Sidak's post-hoc comparisons revealed a significant increase after ethanol binge cycles ($P = 0.0011$), but not after abstinence. AST and ALT levels showed a similar pattern, with a significant increase after binge ethanol intoxication (AST: $P = 0.0009$; ALT: $P = 0.0442$) and maintenance of these elevated levels during abstinence (AST: $P = 0.0049$; ALT: $P = 0.0060$). These results suggest that liver damage persists even after a period of abstinence. The AST/ALT ratio was significantly elevated only after binge drinking (Sidak post hoc, $P = 0.0407$).

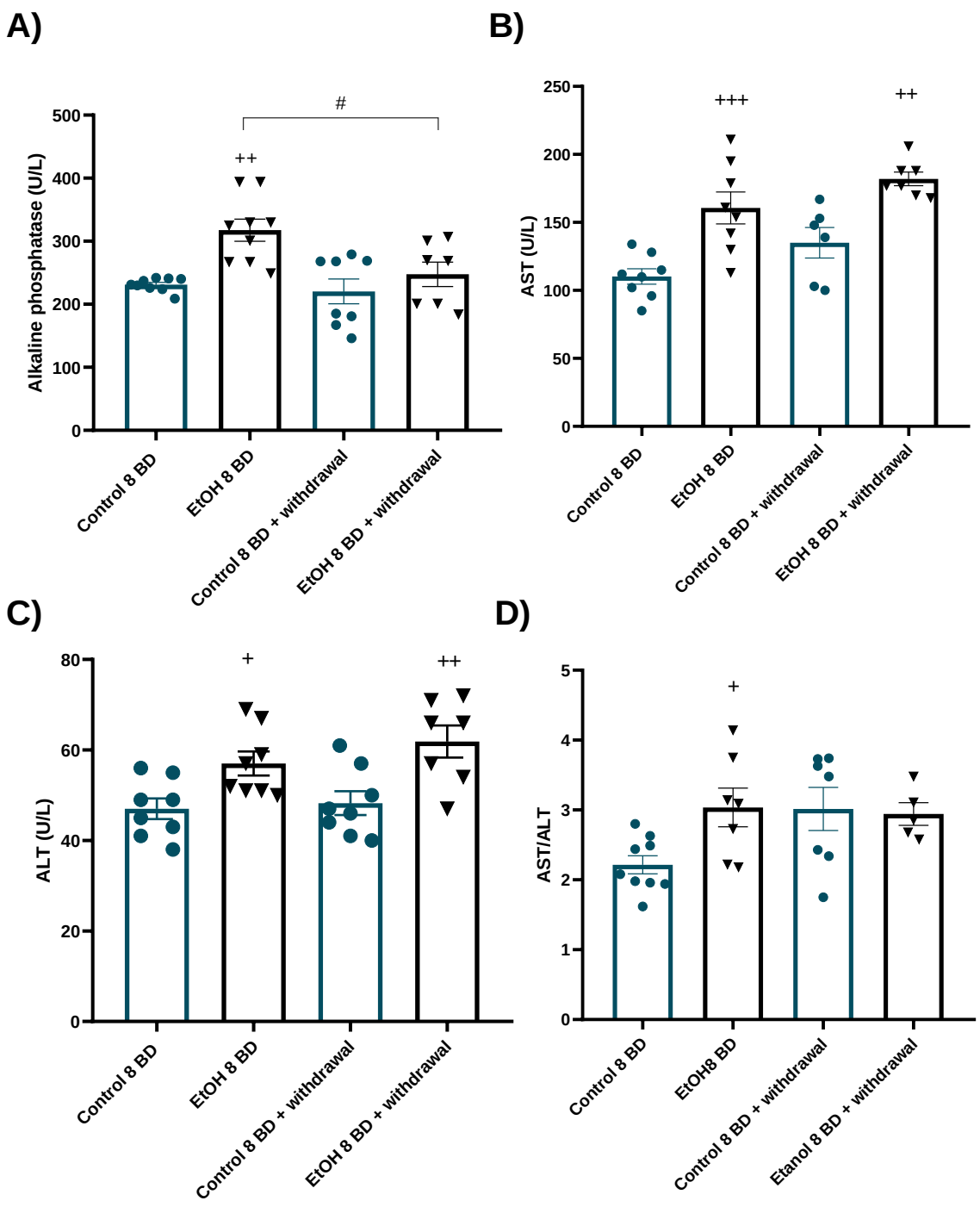

Figure 2: Biochemical changes induced by binge ethanol intoxication in adult female rats. Illustration showing serum levels of alkaline phosphatase (A), AST (B), ALT (C) and AST/ALT

(D) in serum. The data shown are the mean ± SEM of n= 6 - 9 animals/group One-way ANOVA with Sidak post-test. ++P < 0,01 +++P <0,001; +P <0,05, when compared to the respective control; #P<0,05 when compared to the EtOH groups of each period.

Ethanol withdrawal alters lipid metabolism in adult female rats treated with ethanol in a binge pattern.

Figure 3 shows the changes in lipid activity in liver tissue, assessed by triglyceride and VLDL cholesterol levels in response to ethanol treatment. The one-factor analysis of variance revealed that ethanol consumption led to a significant increase in triglyceride levels, suggesting a direct effect of ethanol on lipid metabolism (Sidak post hoc, (P<0.0001). The values remained high even after abstinence from alcohol (P<0.0001). Therefore, the comparison between the ethanol-treated groups confirmed this increase in triglyceride concentration (P=0.0232). With regard to VLDL cholesterol, the group exposed to alcohol showed a change in the dysfunction of lipoprotein metabolism in both the immediate and delayed assessments (<0.0001 in both groups). These results may be correlated, since VLDL is responsible for transporting triglycerides in the blood, and its elevation may be associated with an increase in triglyceride synthesis in the liver, aggravating the risk of liver disease.

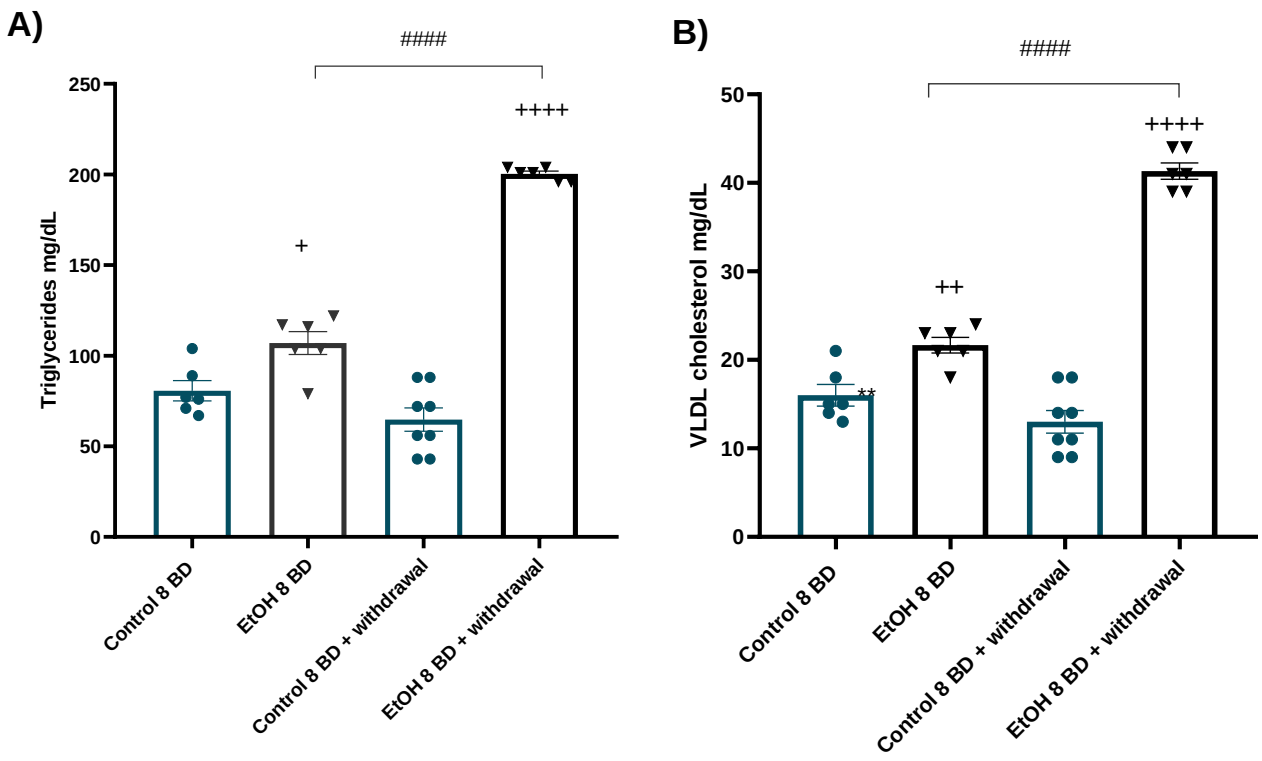

Figure 3: Lipid changes caused by ethanol intoxication in adult female rats. Illustration showing serum levels of triglycerides (A), VLDL cholesterol (B), activity in the serum of the animals. The data shown are the mean ± SEM of n= 6 - 8 animals/group. One-way ANOVA, with Sidak post-test. +P<0,05; ++++P = 0.0001; ++P < 0.01, when compared to the respective control; ####P = 0.0001, when compared to the EtOH groups of each period.

Immediate effects of binge drinking evidenced in the histology of adult rat tissues.

Histological analysis of the livers of the animals treated with alcohol showed significant changes in the liver parenchyma when compared to their respective controls. These showed hepatic steatosis and hepatocellular damage, which were partially reversed in some animals after a period without any administration (Figure 4; Figure 5).

Figure 4 shows the histological findings of animals treated with alcohol or distilled H20. The animals in the control group (A, B, C and D, figure 4) were used as a reference for the normal cytological and histological characteristics of the liver. The photomicrographs (A and B, figure

4) show the normal liver parenchyma, with no evidence of acidophilic or basophilic morphological changes in the liver cells. In photomicrograph C (Figure 4), the triangles indicate the hepatocytes, whose nuclear and cytoplasmic characteristics confirm the normality observed.

In addition, in images A, B, C and D (Figure 4), the black arrows highlight the Kupffer cells, whose nuclear morphological characteristics are also within normal standards. In photomicrograph D (figure 4), the jagged arrow points to a centrilobular vein, showing normal histological patterns and no infiltration of blood cells. In addition, liver structures such as sinusoidal capillaries, blood vessels and portal canals (portal triads) were also preserved, corroborating the normality of the liver parenchyma.

However, the group treated with ethanol (E, F, G and H; figure 4) showed significant changes in their photomicrographs. In image E (figure 4) the liver parenchyma shows notable histopathological changes, where the triangles indicate hepatocytes with hyperchromatic nuclei and intense cytoplasmic acidophilia, characteristics suggestive of cell necrosis. A hexagon within a circle in this same image highlights an area of tissue necrosis. Photomicrograph F (figure 4) shows a portal triad delimited by a dashed ellipse, where intravascular inflammatory infiltrates and necrosis are seen (indicated by a hexagon in the lumen of the portal vein branch), as well as perivascular infiltrates (indicated by a four-pointed star). An additional detail of this image, inserted at the bottom (Insert), highlights the vascular and perivascular infiltrates more clearly. In image G (figure 4), to the left of the dotted demarcation (X), a large number of hepatocytes can be seen with nuclear and cytoplasmic changes, also indicative of necrosis. Finally, in photomicrograph H (figure 4), it is possible to see cells in the process of moderate steatosis (grade 2), where a dotted ellipse delimits a group of these cells, shown in detail in the insert.

These histopathological findings, which include cell/tissue necrosis, vascular (portal) and perivascular infiltrates, as well as steatosis, are indicative of alcoholic liver poisoning.

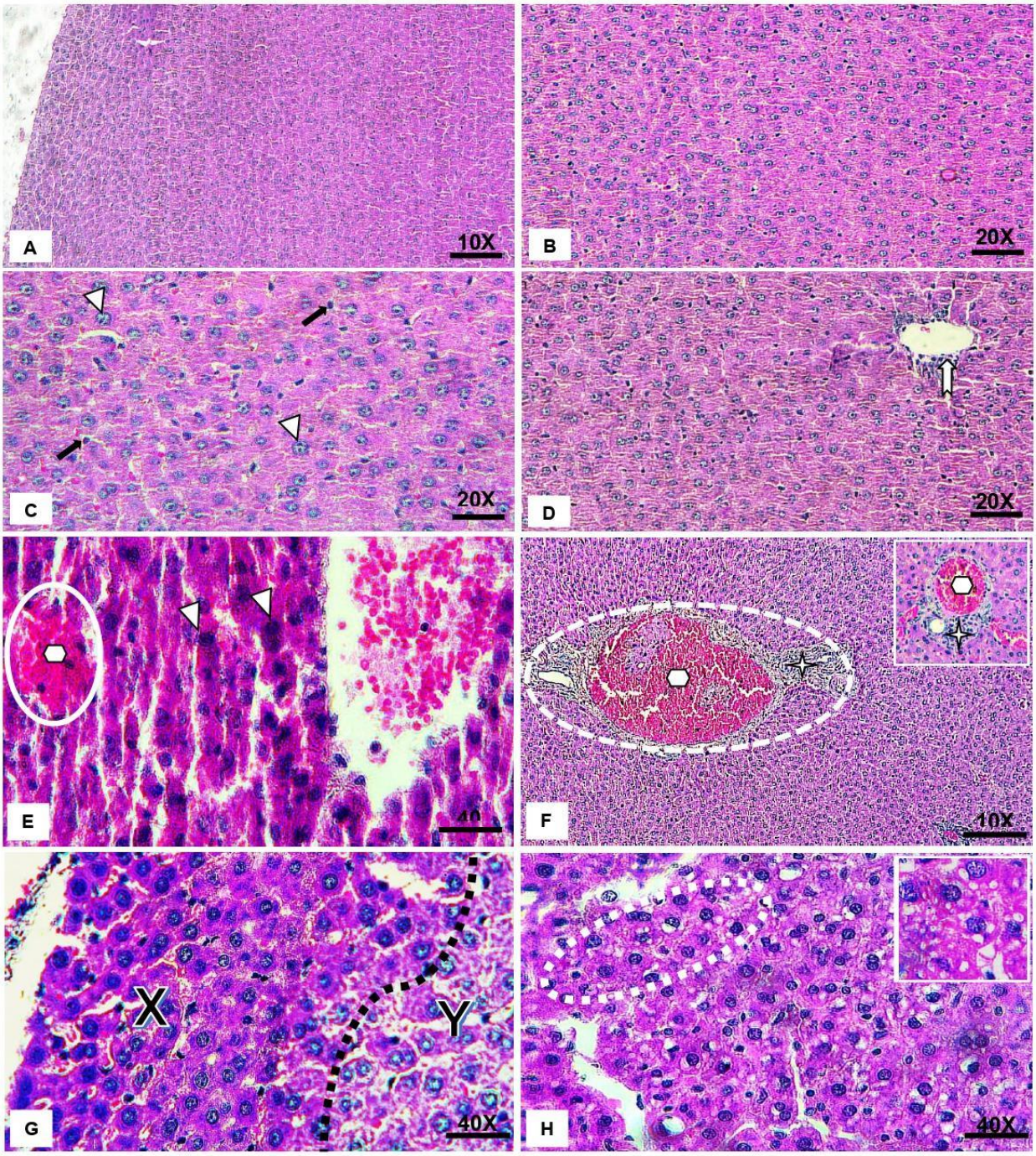

Figure 4: Hepatic photomicroscopy of animals administered distilled EtOH/H2O after 8 treatment cycles. The histological sections of liver tissue from the control group are shown at magnifications of 10x (A) and (B) and 20x (C) and (D). The sections of the tissues intoxicated with alcohol are shown at magnifications of 10x (E) and (F) and 40x (G) and (H).

Long-lasting effects of compulsive consumption observed in the histology of adult rat tissues, even after cessation of use.

Image 5 shows the histopathological findings of the animals 14 days after the last alcohol intoxication. The control group (A, B, C and D) was used as a reference to establish the cytological and histological normality of the liver. Photomicrographs A and B (figure 5) show the normal liver parenchyma, with no acidophilic or basophilic morphological changes in the liver cells. In photomicrograph B (figure 5), the black arrows indicate sinusoidal capillaries of normal dimensions, reinforcing the healthy state of the tissue. In photomicrographs C and D (figure 5), the triangles highlight the hepatocytes, whose nuclear and cytoplasmic characteristics corroborate the normality observed. In these same images, the dotted circles indicate Kupffer cells, whose nuclear characteristics are within normal standards. The white arrows point to sinusoidal capillaries with morphology compatible with normality, confirming the integrity of the liver tissue.

Significant histopathological changes were observed in the animals intoxicated with 8 binge cycles (E, F, G, H, I and J). Photomicrograph E (figure 5) shows dashed ellipses indicating clusters of hepatocytes with hyperchromatic nuclei and intense cytoplasmic acidophilia, both indicative of cell necrosis. In addition, the five-pointed star highlights an inflammatory infiltrate present in the central vein. In photomicrograph F (figure 5), the dotted area delimits a portal triad with intravascular inflammatory infiltrate (five-pointed star) in the portal vein and perivascular infiltrate (black arrow), suggesting a significant inflammatory process.

Photomicrograph G (figure 5) shows hepatocytes with intense cytoplasmic acidophilia and pyknotic nuclei, clear signs of necrosis (indicated by the jagged arrows). The white star in this image indicates vascular infiltrates. In the detail (Insert), the dashed circle delimits a nest of hepatocytes in necrosis and intravascular infiltrate (star), reinforcing the presence of tissue damage. In photomicrograph H (figure 5), a dashed ellipse delimits a group of hepatocytes in necrosis, while the jagged arrows indicate cells in a necrotic process, with pyknotic nuclei. The triangles show macrophages located in the lumen of a portal vein, suggesting an increased immune response.

Photomicrograph I (figure 5) shows a significant reorganization of the liver tissue, with no apparent necrosis. The ellipse outlines a central lobular vein without inflammatory infiltrates, indicating a possible process of tissue recovery. Photomicrograph J confirms that some animals in this group showed signs of tissue reconstitution. In this image, the four-pointed star indicates a central vein without inflammatory infiltrates, while the sinusoidal capillaries (black arrows) maintain normal dimensions, and the Kupffer cells (short notched arrows) show morphology compatible with normality. The hepatocytes (long notched arrows) show apparently normal nuclear and cytoplasmic patterns, with slight steatosis present in some cells.

These findings suggest that the 14-day period of alcohol abstinence may have allowed cytological and histological recomposition of the liver, with a return to normal patterns in some animals in the EtOH 8BD + withdrawal group. On the other hand, histopathological comparisons between the animals in the EtOH 8BD + withdrawal group and those in the EtOH 8BD group without withdrawal showed that the histopathological damage was more intense and extensive in the animals that did not undergo the withdrawal period.

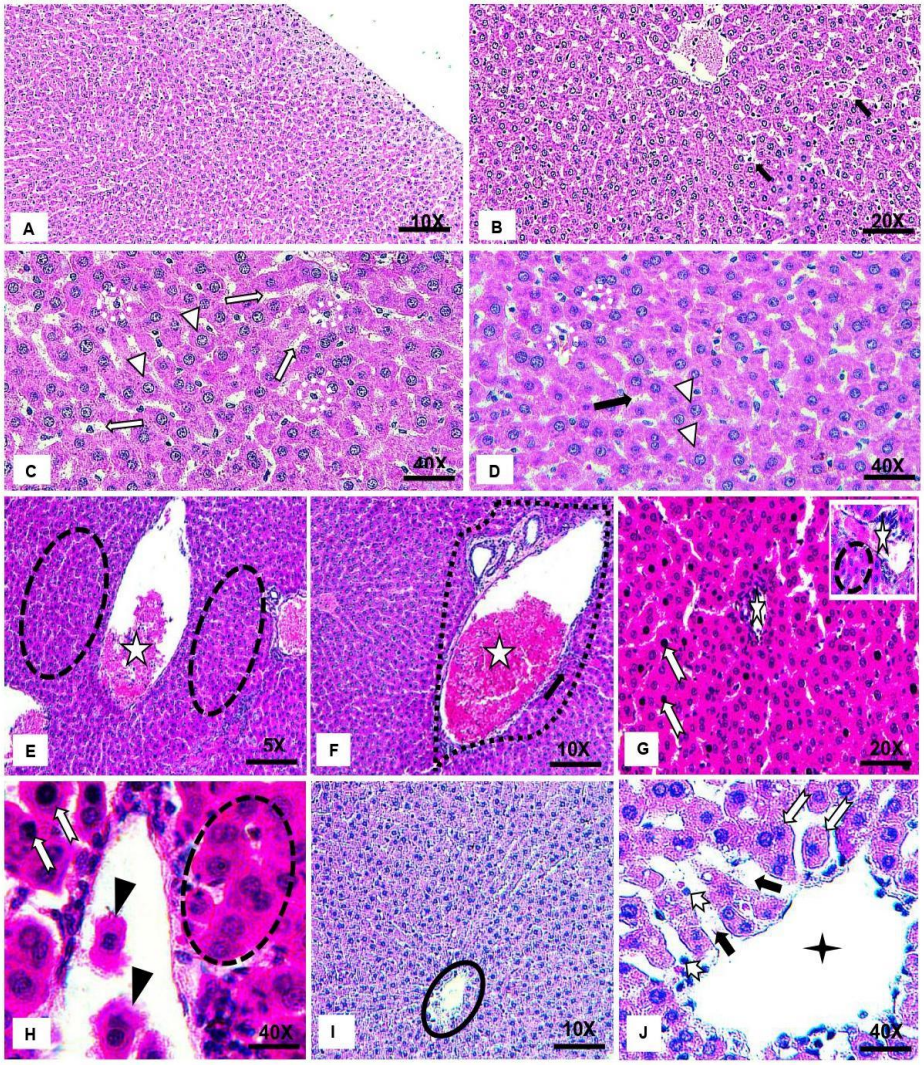

Figure 5: Hepatic photomicroscopy of animals administered distilled EtOH/H2O after 8 cycles of treatment, followed by a period without any administration. The histological images of the control group were examined at 10x (A), 20x (B), and 40x (C and D) magnifications. The alcohol-treated groups were observed at 5x (E), 10x (F and I), 20x (G), and 40x (H and J) magnifications.

Binge ethanol treatment in adult rats induces cumulative effects on oxidative damage in liver tissue

Figure 6 illustrates the alterations in the oxidative activities of liver tissue, assessed by MDA, catalase, and SOD levels, in response to ethanol treatment. One-way analysis of variance revealed a significant increase in MDA levels after binge ethanol cycles, which persisted even after drug

withdrawal (Sidak's post hoc, P < 0.0001 in both groups). Comparison between ethanol-treated groups confirmed this elevation in MDA levels. Regarding antioxidant enzymes, catalase activity showed a significant increase after ethanol intoxication, with maintenance of these elevated levels during the abstinence phase (Sidak's post hoc, P = 0.0029 and P = 0.0003, respectively). Furthermore, catalase activity was higher during abstinence compared to the immediate evaluation period after the 8 cycles (Sidak's post hoc, P = 0.0027). Similarly, SOD levels also increased significantly both after alcohol treatment and after drug withdrawal (Sidak's post hoc, P = 0.0068 and P = 0.0006, respectively), although no difference was observed between the ethanol-treated groups.

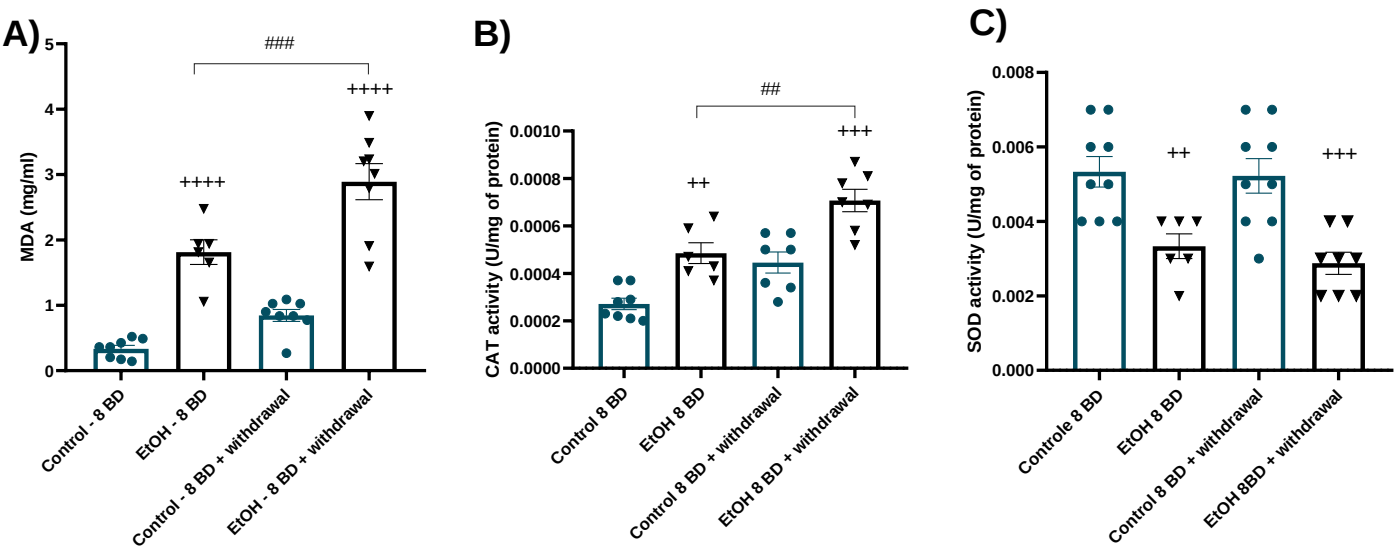

Figure 6: Oxidative alterations induced by intermittent and episodic ethanol treatment in adult rats. Illustration showing serum MDA levels (A), catalase enzyme activity (B), and SOD enzyme activity (C) in liver tissue. Data are presented as mean ± SEM, n= 6 - 9 animals/group. One-way ANOVA with Sidak's post-hoc test was used. ++++P = 0.0001; ++P < 0.01; +++P < 0.001, when compared to the respective control; ###P < 0.001; ##P < 0.01, when compared to the EtOH groups of each period.

Discussion

The present study demonstrated that binge ethanol consumption in adult rats can induce oxidative hepatic alterations that persist even after substance withdrawal. These alterations also

reflect on the enzymatic function and morphology of the intoxicated liver, contributing, in part, to the development of alcoholic liver disease ALD.

The mechanisms involved in the development of ALD are complex and encompass a spectrum of liver damage, which can manifest as steatosis, steatohepatitis, fibrosis, cirrhosis, and, at more advanced stages, hepatocellular carcinoma (Osna et al., 2022; Lai et al., 2024). This scenario highlights how excessive alcohol consumption can induce progressive pathological processes in the liver, especially through the action of ethanol and its metabolites, which, when accumulating in the tissue, induce alterations in lipid and oxidative metabolism. These processes are directly related to ethanol metabolism, which is converted into acetaldehyde - a highly reactive compound, more toxic than alcohol itself (Stevens et al., 1981; Cederbaum, 2012). The presence of this, together with the production of reactive oxygen species (ROS), contributes significantly to the development of oxidative stress (Cederbaum, 2012; Mansouri et al., 2018; Holbrook et al., 2023). This imbalance in the redox system is a trigger for cellular damage, including mitochondrial dysfunction, DNA damage, and increased lipid peroxidation, compromising the integrity and functionality of liver tissue (Tell, Vascotto & Tiribelli, 2013; Mansouri et al., 2018).

The investigation of serum biochemical parameters plays a fundamental role in the diagnosis and monitoring of liver diseases, since it allows the detection of early liver lesions (Woreta & Alqahtani, 2015). Among these parameters, the enzymatic evaluation of markers such as aminotransferases (AST and ALT) and alkaline phosphatase is widely used as an indicator of hepatocellular damage. Therefore, the elevation of these enzymes is frequently observed in studies that induce hepatic alterations by excessive alcohol consumption (Carson et al., 1996; Bertola et al., 2013a; Bertola et al., 2013b; Neyrinck et al., 2017).

Furthermore, as expected, the binge ethanol protocol applied in this study revealed a significant increase in ALT and AST levels 24 hours after the last binge episode. Interestingly, these levels remained elevated even after 14 days of alcohol abstinence. This fact can be attributed to the fact that, in the development of ALD, AST tends to be higher than ALT, due to ethanol damage to the liver mitochondria, where AST is more concentrated. Additionally, mitochondrial AST has a longer half-life, which may justify the persistence of elevated levels of this enzyme

even after cessation of alcohol consumption (Woreta & Alqahtani, 2015; Harris et al., 2021; Thomes et al., 2021). Another factor also suggests that the presence of inflammation and liver fibrosis processes can increase the period of elevation of aminotransferases, even after cessation of alcohol, while the liver recovers from chronic oxidative damage (Cuperus, Drenth and Tjwa, 2017). This mechanism may explain the persistence of elevated AST and ALT levels in our study, even after the abstinence period.

Another relevant parameter in the evaluation of liver diseases is the AST/ALT ratio. This index helps to differentiate between non-alcoholic steatohepatitis (NASH) and alcoholic liver disease (ALD). In NASH, this ratio tends to be less than 1, while in ALD it is greater than 1, and is usually greater than 2 (Zamin et al., 2002; Woreta & Alqahtani, 2015). In our results, significance was observed in the AST/ALT ratio only in the immediate post-consumption evaluation, but not after abstinence. This may indicate a progressive inversion of the ratio as the liver recovers, as observed in clinical studies (Woreta & Alqahtani, 2015).

As a complementary assessment, we observed an increase in serum levels of alkaline phosphatase (ALP), which, although not a specific marker of hepatocellular damage, may indicate biliary involvement in ALD. These data indicate a more comprehensive and advanced picture of liver injury, as it affects both hepatocytes and bile ducts. The increase in ALP may be a consequence of obstruction or inflammation in the bile ducts, with damage to the cells that line them, which often accompanies cholestatic and inflammatory processes in more severe liver diseases (Tung & Carithers, 1999). However, further studies are needed to understand the mechanisms involved in this process.

Based on these findings, understanding steatosis, or fatty liver, as one of the first signs of liver injury associated with alcohol abuse, is essential to contextualize the impact of oxidative stress on the progression of ALD. Steatosis is the first response of the liver to alcohol abuse and is the initial stage of ALD, being completely reversible with alcohol abstinence (Gao et al., 2011; Abenavoli et al., 2016; Parola and Pinzani, 2019; Kong et al., 2019). This picture is the result of excessive accumulation of lipids in hepatocytes, triggered by an imbalance in fatty acid metabolism and aggravated by the generation of ROS during ethanol metabolism (Baraona &

Lieber, 1979; Orman, Odena & Bataller, 2015). Such a process impairs mitochondrial oxidation of lipids, increasing triglyceride synthesis and contributing to fat accumulation in the liver. If untreated, steatosis can progress to more severe stages, such as steatohepatitis, fibrosis, and eventually cirrhosis (Kong et al., 2019).

Indeed, the increase in triglyceride levels is strong evidence of the relationship between excessive ethanol consumption and lipid imbalance. Alcohol intoxication significantly affects hepatic metabolism, influencing processes such as increased fatty acid uptake by the liver, reduced mitochondrial β-oxidation, and decreased secretion of very low-density lipoprotein (VLDL) (Hyun et al., 2021). These mechanisms lead to the accumulation of triglycerides in the liver and circulation, contributing to dyslipidemia (Kong et al. 2019).

As expected, our results showed increased serum levels of triglycerides and VLDL after alcohol intoxication. These findings are consistent with studies that use ethanol as the sole agent to induce hepatic alterations, without the need for additional insults (Cresci et al., 2014; Gao et al., 2015; Williams et al., 2015). These studies suggest different mechanisms responsible for the increase in triglycerides, including the upregulation of the CYP2E1 enzyme (Yang et al., 2012; Wu et al., 2012; Zhou et al., 2014), the activation of lipogenic gene expression, such as SREBP (Yang et al., 2012; Chen et al., 2015), and the reduction in the activity of the peroxisome proliferator-activated receptor α (PPARα) (Desai et al., 2017; Williams et al., 2015), which plays an essential role in the regulation of fatty acid metabolism.

Curiously, the elevated levels of triglycerides and VLDL serum did not return to normal levels even after 14 days of alcohol abstinence. These findings diverge from studies that report that alcohol abstinence usually effectively restores hepatic lipid metabolism and reverses liver lesions and inflammation induced by chronic alcohol consumption (Thomes et al., 2019; Pi et al., 2021).

Data like this suggest that to better understand these mechanisms, it is necessary to observe the effects of abstinence for a longer period, in order to clarify the complete timeline of liver regeneration and its possible variations according to the mode of consumption. It is important to

emphasize that, although inflammation and some liver damage may begin to be reversed after about one week of abstinence, mitochondrial dysfunctions and triglyceride accumulation tend to persist (Pi et al., 2021). These dysfunctions, resulting from the downregulation of fatty acid β-oxidation and decreased VLDL secretion, can prolong the elevation of triglyceride levels in the blood for several weeks after cessation of alcohol consumption (Kong et al., 2019; Pi et al., 2021).

Having observed the increase in AST and ALT levels, which are indicators of hepatocellular damage, we complemented the investigation with a histological analysis to directly observe the structural changes in liver cells This approach is essential for studying hepatic, as it enables a detailed assessment of morphological alterations in hepatocytes and provides insights into alcohol-induced lesions (European Association for the Study of Liver, 2012). Our findings confirmed the presence of significant histopathological alterations, including cellular necrosis and grade 2 hepatic steatosis, observed immediately after acute alcohol intoxication. These alterations are consistent with previous studies, which indicate that excessive alcohol consumption can result in the accumulation of lipids in the liver and trigger cellular lesions, which, if untreated, can progress to more severe forms of liver disease (Zhou et al., 2014; Williams et al., 2015; Neyrinck et al., 2017; Iracheta-Vellve et al., 2018). In addition, histological studies indicate that the initial phases of ALD present alterations including an increase in the diameter of central veins and bile ducts, indicating a decrease in blood supply, as also observed in our results (Didenko et al., 2023).

Furthermore, hepatic fibrosis was observed in some animals, a key condition in chronic liver diseases (Schuppan, 2016). In the context of progressive fibrosis, ethanol intoxication can induce the transformation of hepatic stellate cells, normally found in an inactive state, into activated myofibroblasts (Parola and Pinzani, 2019). These myofibroblasts produce excessive amounts of extracellular matrix, replacing healthy liver parenchyma and altering the tissue architecture. As a result, there is a progressive impairment of liver function as fibrosis progresses, aggravating the clinical picture (Bataller & Brenner, 2001; Seitz et al, 2018). Although experimental models have advanced significantly, it is important to recognize that current protocols still cannot fully reproduce the pathological picture of human ALD.

Our results demonstrate that, although the lesions developed with less intensity, they remained elevated during the abstinence period, evidencing the need for more detailed investigations to understand the recovery mechanisms under the binge ethanol pattern applied. It is important to highlight that our experiments were performed on female rats, which have a greater susceptibility to alcohol-induced liver injury (Iimuro et al., 1997; Yin et al., 2003; Fulham & Mandrekar, 2016). In addition, intoxication was initiated during the phase corresponding to the adolescence of the animals, a period in which the liver is more susceptible to damage, since this organ is in the process of maturation and presents metabolic and structural characteristics different from a fully developed adult liver (Skala & Walter, 2013). These factors may have contributed to the severity of the data obtained and influenced the results obtained.

In this context, our results demonstrated significant oxidative alterations in the liver of rats subjected to binge-like alcohol consumption. In fact, after 8 cycles of ethanol intoxication, an increase in MDA levels was observed in the liver tissue, a toxic byproduct resulting from lipid peroxidation. Notably, this increase remained elevated even after a period of abstinence, suggesting the persistence of long-term liver damage. These findings corroborate the results of Fernandes et al. (2018), who used a similar protocol, although with a smaller number of intoxication cycles and with adolescent animals. Nevertheless, the authors reported a significant increase in lipid peroxidation levels in immediate assessments, indicating that even a lower exposure to ethanol can induce acute oxidative damage in young livers (Fernandes et al., 2018). It should be emphasized that the increase in markers of lipid peroxidation induced by binge ethanol, such as MDA, is widely described in the literature as an indicator of oxidative stress in liver tissue (Demeilliers et al., 2002; Cederbaum et al., 2009; Abdelmegeed et al., 2013; Yang et al., 2012; Yang et al., 2014; Li et al., 2017; Ramirez et al., 2017).

Equally important, our investigation demonstrated a sustained increase in MDA levels after ethanol abstinence, diverging from studies that suggest a reversal of MDA levels after discontinuation of alcohol consumption, as observed in models of chronic alcohol feeding followed by acute binge episodes (Kang et al., 2022). On the other hand, our findings align with studies that report that one week of abstinence was not sufficient to reverse the excessive

formation of MDA induced by chronic alcohol feeding (Pi et al., 2021). These reports suggest that the persistence of damage may be associated with factors such as the intensity and duration of ethanol exposure, as well as the regenerative capacity of liver tissue (Bertola et al., 2013; Mathews et al., 2014; Dastidar et al., 2018; Torres-Magno et al., 2024).

Furthermore, we observed variations in the activity of antioxidant system components, with a significant increase in CAT activity in the liver after alcohol treatment. This finding can be interpreted as an adaptive response of the liver tissue to increased lipid peroxidation, aiming to mitigate oxidative damage induced by the accumulation of reactive oxygen species (ROS) (Oshino et al., 1973; Anwar et al., 2024). Interestingly, CAT activity remained elevated even after 14 days of alcohol abstinence, suggesting a persistence of redox imbalance. Similar findings were reported in clinical studies with alcoholic patients, in which CAT activities did not return to normal levels after one week of abstinence (Peng et al., 2005). These data suggest a close relationship between oxidative alterations found during abstinence and the extent of lipid peroxidation (Peng et al., 2005; Huang et al., 2009).

Our results also demonstrated a reduction in SOD enzymatic activity in adult rats intoxicated with alcohol, both in immediate assessments and after a period of abstinence. These findings are important as they suggest an oxidative imbalance associated with ethanol toxicity, which induces the depletion of fundamental antioxidant enzymes (Das et al., 2007; Leung & Nieto, 2012). The decrease in SOD activity, an enzyme responsible for the dismutation of superoxide radical into hydrogen peroxide, induced by acute alcohol consumption is widely reported in the literature (Zeng et al., 2017; Fernandes et al., 2018; Yuan et al., 2018). This fact is frequently associated with the accumulation of ROS and the consequent increase in lipid peroxidation, which was also corroborated by our results (Li et al., 2015; Wang et al, 2017; Singh et al., 2020). This picture of decreased SOD enzymatic activity, combined with increased ROS production, suggests an oxidative imbalance that prolongs liver damage, even after cessation of alcohol consumption. In view of this, the findings reinforce the hypothesis that liver regeneration mechanisms may not be sufficient to reverse the chronic oxidative damage induced by excessive

ethanol consumption, contributing to the progression of long-lasting liver lesions (Cordero-Espinoza & Huch, 2018; Pibiri & Simbula, 2022).

After observing the structural changes in the liver, we focused our analysis on investigating the role of oxidative stress as one of the underlying mechanisms of alcohol-induced lesions. Oxidative imbalance plays a central role in the progression and complications of ALD, as it causes an oxidative disruption in liver cells, contributing to inflammatory and apoptotic processes, as well as accelerating the development of liver lesions (Ceni et al., 2014; Michalak et al., 2021).

Damage caused by ethanol intoxication is intimately related to acetaldehyde, which ends up damaging cellular structures such as DNA, lipids, and proteins, as well as interfering with crucial processes such as lipid metabolism and cellular respiration (Cederbaum et al., 2015). These, in turn, trigger inflammation, increased fibrogenesis, and apoptotic processes, which can result in progressive liver lesions (Ceni et al., 2014; Cederbaum et al., 2015; Sugimoto & Takei, 2016; Chao et al., 2018).

As the literature shows, the induction of liver injury exclusively by alcohol tends to generate mild hepatic alterations, which reflect the initial stages of ALD in humans (Mathews et al., 2014). However, there is a wide variety of experimental protocols that use different alcohol administration regimens, which can produce damage of different intensity, even in the initial stages of ALD (Torres-Magno et al., 2024).

Conclusions

Based on the findings of this study, it can be concluded that excessive ethanol consumption triggered an oxidative imbalance significantly associated with the development of hepatocyte damage and hepatic steatosis. Although the literature suggests that alcohol abstinence can reverse liver damage and restore lipid metabolism, our experiments showed that markers of injury, oxidative stress, and steatosis remained elevated even after 14 days of abstinence. Histological analysis corroborated these results, evidencing grade 2 hepatic steatosis and cellular necrosis, typical alterations of ALD.

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All authors contributed to the data analysis of this study.

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Conflict of Interest:

The authors declare that there is no conflict of interest.

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3. Considerações finais

O consumo excessivo de álcool, em especial o *binge drinking* entre adolescentes, representa um sério problema para a saúde pública global. Nossos estudos evidenciaram que ciclos repetidos de *binge* EtOH induz alterações significativas no fígado, manifestando-se como esteatose, necrose e aumento da peroxidação lipídica, mesmo após períodos de abstinência. Os dados obtidos em nossa análise experimental, aliados à revisão bibliométrica de literatura, ressaltam a íntima relação entre o consumo episódico de álcool e processos patológicos como estresse oxidativo e disfunção lipídica.

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5. **Anexos**

Anexo I. Comprovação de submissão

Pharmacological Research

What is known about binge drinking and liver function? A bibliometric approach of the 100 most cited articles, past and future trends

--Manuscript Draft--

Manuscript Number:	
Article Type:	Review Article
Keywords:	Binge drinking, liver changes, hepatoxicity, oxidative stress, alcohol consumption
Corresponding Author:	Luanna Melo Pereira Fernandes, Ph.D University of Pará State Belém, Pará BRAZIL
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	Jofre Jacob da Silva Freitas
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	Enéas de Andrade Fontes-Júnior
	Rafael Rodrigues Lima
	Cristiane do Socorro Ferraz Maia
	Luanna Melo Pereira Fernandes, Ph.D
Abstract:	Alcohol consumption has been culturally accepted for centuries due to its psychotropic effects and legal status. Alcohol intake, mostly in a binge-like manner, has demonstrated several risks for the development of addiction, immunological disturbances, neurophysiological insults, and particularly hepatotoxicity. We conducted a bibliometric analysis of the 100 most cited articles related to binge drinking and hepatotoxicity. We retrieved articles included in the criteria from the Web of Science Core Collection (WoS-CC) database, following the bibliometric rules, such as number of annual publications and citations, authors' productivity, keyword analysis, journals of publication, geographic distribution, and funding agencies. Critical analysis focused on study types and binge-type protocol. Results indicated Gao B as the most prolific author, with 18 publications and 2,559 citations. The journal with the most papers published was Hepatology, and the more frequent keywords were "liver steatosis" and "alcohol". Geographic aspects, the United States was the prominent country of publications, with 68 articles and 7,438 citations. The analysis of funding agencies confirmed that the National Institutes of Health (NIH) stood out as the largest scientific funder. Content mapping identified five categories of binge-like ethanol protocols, with "binge ethanol" and "chronic plus binge ethanol feeding" as the most common models for inducing hepatotoxicity. Our data highlighted the scenario of the most cited scientific papers related to binge drinking and liver dysfunction, evidencing that the extent and mechanisms of liver damage vary depending on the binge drinking pattern, with inflammatory processes, oxidative stress, and lipid metabolism dysregulation the main source of hepatotoxicity.
Suggested Reviewers:	Rosana Camarini camarini@icb.usp.br
	Rodrigo Cunha

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Anexo II. Certificado de aprovação na Comissão de Ética em Uso de Animais – UEPa/ CCBS.

UNIVERSIDADE DO ESTADO DO PARÁ
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
COMISSÃO DE ÉTICA EM USO DE ANIMAIS

ANEXO III

CERTIFICADO

Certificamos que a proposta intitulada **"EFEITO DO METABOLISMO DO ETANOL NO PADRÃO BINGE DA ADOLESCÊNCIA À VIDA ADULTA E SUAS REPERCUSSÕES HEPÁTICAS"**, registrada com o nº 16/2023, sob a responsabilidade de LUANNA DE MELO PEREIRA FERNANDES - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei nº 11.794, de 08 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal - CONCEA, e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) da UNIVERSIDADE DO ESTADO DO PARÁ, em 07 / 06 / 2023, *ad referendum*.

Finalidade	( ) Ensino (X) Pesquisa Científica
Vigência da autorização	06/2023 à 06/2024
Espécie/linhagem/raça	*Rato heterogênico / Rattus norvegicus* / Wistar
Nº de animais	117
Peso/Idade	60-70g / 30 dias
Sexo	Fêmeas
Origem	Biotério do Instituto Evandro Chagas

Belém, 07 de junho de 2023.

Prof. Dr. Rodrigo Santiago Barbosa Rocha
Coord. do CEUA/CCBS/UEPA