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**Efeitos da cafeína na toxicidade e na
autoadministração de álcool em ratos**

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RESUMO

A cafeína e o álcool são algumas das substâncias psicoativas mais utilizadas no mundo, que são conhecidas por serem usadas em conjunto extensivamente. Sabe-se que o álcool altera a comunicação entre neurônios do sistema excitatório e inibitório do cérebro, sua principal ação é mediada pelos neurotransmissores glutamato, ácido gama-aminobutírico (GABA) e também dopamina. Além disso, o álcool pode promover a sinalização da adenosina, aumentando sua síntese e inibindo seu transportador. A cafeína, por sua vez, é um estimulante, com seu principal alvo de ação no sistema adenosinérgico, com ação antagonista não seletiva de receptores de adenosina. Não é incomum o uso de álcool com bebidas cafeínadas. No entanto, pouco se sabe sobre o grau em que eles podem potencializar mutuamente o consumo um do outro e o resultado dessa coexposição a longo prazo. Também o álcool é capaz de lesionar constituintes celulares essenciais, aumentando o dano ao DNA no cérebro. Aqui, nosso objetivo é investigar o comportamento de autoadministração mista de álcool e cafeína. Além disso, o do índice de danos no DNA resultante da neurotoxicidade do álcool no córtex pré-frontal (CPF).

Palavras-chaves: Álcool, Abstinência ao álcool, Autoadministração, Manifestações comportamentais animais, Dependência alcoólica, Cafeína, Dano de DNA.

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1. INTRODUÇÃO

1.1 Álcool

1.1.1 Aspectos gerais

Dados epidemiológicos apontam que consumo de álcool continua sendo um grande problema de saúde pública, chamando atenção tanto em termos de políticas públicas e de instituições como a Organização Mundial de Saúde (OMS) e a Organização PanAmericana de Saúde (OPAS). Em 2016, mais de 2,3 bilhões de pessoas, com 15 anos ou mais, utilizaram bebidas alcoólicas rotineiramente. Nas Américas, Europa e Pacífico Ocidental, o álcool foi consumido por mais da metade da população. Cerca de 26% dos adolescentes (15 a 19 anos) do mundo utilizam frequentemente o álcool, havendo na Europa a maior prevalência de consumo de álcool entre adolescentes, com 44%, seguidas pelas Américas, e a Região do Pacífico Ocidental, ambas com 38%. A média mundial de consumo de álcool, no ano de 2016, foi de 6.4 L de álcool per capita (pessoas com 15 anos ou mais) (WORLD HEALTH ORGANIZATION; MANAGEMENT OF SUBSTANCE ABUSE TEAM; WORLD HEALTH ORGANIZATION, 2018). No Brasil, o consumo é de 7,8 L de álcool per capita, com estimativa que no ano de 2025 o consumo total de álcool puro per capita seja de 8,3 L. Um dado alarmante aponta que destes, 8.4% da população preenche critérios de abuso ou dependência, segundo a OMS (WORLD HEALTH ORGANIZATION; MANAGEMENT OF SUBSTANCE ABUSE TEAM; WORLD HEALTH ORGANIZATION, 2018). Sabe-se que as implicações deste uso não afetam somente a saúde dos usuários, mas acarretam também diversos problemas sociais, como aumento no número de acidentes de trânsito, relações sexuais desprotegidas, e violência (UNODC, 2017).

Proveniente do produto da fermentação de frutas, cereais ou da hidratação do etileno, o Álcool (Etanol) é uma molécula orgânica simples composta de um grupo hidroxila, uma cadeia alifática curta com dois carbonos e representado pela fórmula molecular $\text{CH}_3\text{CH}_2\text{OH}$, os radicais hidroxil e etil conferem propriedades tanto hidrofílicas e lipofílicas. Encontrado em bebidas social e comercialmente aceitas, como a cerveja, vinho e destilados, a principal atuação do álcool é no Sistema Nervoso Central (SNC), sendo classificado farmacologicamente como uma droga

depressora do SNC. No entanto, o etanol pode apresentar efeitos estimulantes, dependendo do tempo e da dose consumida. Consumido em doses baixas, o álcool tem efeito estimulante, causando desinibição, sentimentos de euforia. Já em doses mais altas, tem efeitos depressores e sedativos, levando a incoordenação motora, relaxamento, déficit cognitivo e de memória (BRANDES, 2014; BRUNTON, 2012; GIGLIOTTI; BESSA, 2004; VENGELIENE et al., 2008).

1.1.2 Aspectos neurobiológicos e comportamentais do álcool

O álcool altera a comunicação entre neurônios excitatório e inibitório do sistema nervoso central. Os efeitos do etanol podem causar uma comunicação alterada entre neurônios, por ação direta ou indireta, mediada principalmente pelos neurotransmissores glutamato e ácido gama-aminobutírico (GABA), e também dopamina, acetilcolina e serotonina (WAKITA et al., 2012; ZALESKI et al., 2004). Um dos principais alvos de ação do etanol é a sinapse inibitória GABAérgica (FISCHER; FRANKE; ILLES, 2003; WAKITA et al., 2012). Os receptores GABA se subdividem em GABA_A e GABA_B e o etanol tem maior influência nos receptores GABA_A, que são ionotrópicos e possuem três principais subunidades (α , β e γ), que delimitam canais de cloreto (Cl⁻). Quando o etanol atinge o SNC, seu efeito potencializa a ação do GABA, permitindo o aumento da passagem do Cl⁻ para o interior da célula neuronal, hiperpolarizando-a e ativando seu efeito inibitório (FISCHER; FRANKE; ILLES, 2003; TATEBAYASHI; MOTOMURA; NARAHASHI, 1998). A hiperpolarização do GABA também influencia a liberação de dopamina no sistema mesocorticolímbico, causando processos fisiológicos recompensadores. Além disso, o consumo agudo de álcool inibe o funcionamento da neurotransmissão glutamatérgica excitatória, deprimindo a responsividade dos receptores de glutamato de canal iônico, particularmente os receptores N-metil D-Aspartato (NMDA) (JULIEN; ADVOCAT; COMATY, 2011; MUKHERJEE et al., 2008). O rompimento do funcionamento do receptor NDMA e a regulação positiva (up-regulation) dos receptores NMDA após o consumo crônico, tem sido relacionado com dificuldades de aprendizado e de memória induzidas pelo álcool (JULIEN; ADVOCAT; COMATY, 2011; KOOB et al., 2004; MUKHERJEE et al., 2008).

A ação do etanol sobre o sistema recompensa é relacionada ao aumento da liberação de dopamina no sistema mesolímbico. Os efeitos do álcool na liberação de dopamina podem resultar de efeitos diretos sobre o disparo de neurônios dopaminérgicos na área tegmental ventral (VTA) e / ou ser mediada por interações com outros sistemas de sinalização, tais como os neurotransmissores glutamato, ácido γ -aminobutírico (GABA) e serotonina. Com o tempo, a exposição crônica ao álcool causa algumas mudanças adaptativas, tais como diminuição da liberação de dopamina no sistema mesolímbico e estriado e aumento da transmissão de glutamato (ROSSETTI; CARBONI; FADDA, 1999; WEISS et al., 1996). Essas neuroadaptações parecem desempenhar papéis fundamentais no desenvolvimento da dependência do álcool. Além disto, é provável que diferentes respostas adaptativas ocorram em diferentes indivíduos, devido à grande variabilidade individual destas mudanças adaptativas, que envolvem tanto características genéticas como processos de neuroplasticidade induzidas pelo ambiente, nenhum agente terapêutico é completamente eficaz em todas os indivíduos dependentes de álcool (SPANAGEL; KIEFER, 2008).

Ainda acerca dos efeitos no SNC, além dos efeitos sobre o GABA e Glutamato, o álcool também promove a sinalização indireta da adenosina, aumentando os níveis de adenosina extracelular inibindo seu transportador, o ENT1 (ALLEN-GIPSON et al., 2009; NAGY et al., 1990). Em segundo lugar, o acetato gerado pela metabolização do álcool promove a síntese de adenosina. Este efeito promove um acúmulo de adenosina extracelular, fazendo com que haja uma interação maior da adenosina com seus receptores. Este efeito foi associado à sedação induzida pelo etanol e à incoordenação motora, pois camundongos sem o transportador de adenosina ENT1 exibiram sensibilidade significativamente reduzida a esses efeitos (CHOI et al., 2004).

A síndrome de abstinência é causada pela parada da ingestão crônica do álcool. Os sintomas da abstinência podem ser náuseas, vômitos, dor de cabeça, tremor, humor deprimido, ansiedade, sonolência e diminuição do estado de alerta (MCKINNEY; COYLE, 2006; PENNING et al., 2013), podem ocorrer entre 6 e 48 horas após a última ingestão, e a severidade desses sintomas vai depender do hábito, da quantidade, e tempo de uso (GIGLIOTTI; BESSA, 2004; GRANT, 1998).

As pesquisas neurobiológicas são desenvolvidas com modelos animais que mimetizam diversos aspectos da dependência em humanos, tais como a perda de controle sobre o consumo, *craving* ou fissura e recaída (COLOMBO et al., 2004; GINSBURG; LAMB, 2013). Entre estes modelos, estão incluídos modelos de condicionamento, autoadministração por condicionamento operante ou por livre escolha, além de modelos de abstinência (BONASSOLI; MILANI; DE OLIVEIRA, 2011; CABRAL et al., 2006). Destes, o modelo de autoadministração de álcool por via oral são os mais próximos da forma humana de usar esta substância. O animal deverá executar uma resposta comportamental, ou seja, realizar uma ação como pressionar uma alavanca para receber doses de álcool (LOPEZ; BECKER, 2014). E, nos modelos de abstinência, é possível verificar que os animais em abstinência apresentam manifestações comportamentais semelhantes às manifestações em humanos. Estas, incluem sinais e sintomas como ansiedade, convulsões, hipoatividade, estresse e sintomas tipo depressivos (BALDWIN et al., 1991; CABRAL et al., 2006; DOREMUS-FITZWATER; SPEAR, 2007).

1.1.3 Neurotoxicidade do álcool

Nos últimos anos, a neuroinflamação e o estresse oxidativo têm sido associados a processos de neurodegeneração desencadeados por drogas de abuso, produtos químicos, trauma ou doença (ALFONSO-LOECHES et al., 2010; BLOCK; HONG, 2005). Basicamente, a inflamação envolve a síntese e liberação de mediadores pró-inflamatórios, como citocinas e quimiocinas. A neuroinflamação é relacionada com a ativação da microglia, processo conhecido como gliose, ou seja, as células da glia do cérebro conhecidas por servir tanto como fonte quanto como alvos de mediadores pró-inflamatórios (O'CALLAGHAN; SRIRAM; MILLER, 2008). A microglia é responsável pela resposta imune inata no cérebro. Sua função é realizar manutenção e limpar os restos celulares (BEYER et al., 2000). A microglia ativada sofre alterações morfológicas que mudam da microglia ramificada em repouso para a microglia amebóide ativada (KREUTZBERG, 1996). A ativação de células microgliais que ocorrem após a administração de etanol ativam as vias de sinalização e promovem a liberação de espécies reativas de oxigênio (ERO), pró-mediadores inflamatórios e citocinas, incluindo interleucinas e fator de necrose

tumoral alfa. Um aumento de ERO intracelular ocorre na microglia como resposta à ativação do hidrogênio-fosfato de nicotinamida-adenina-dinucleotídeo-fosfato (NADPH) que também aumenta a produção de fatores pró-inflamatórios neurotóxicos. Todos esses eventos inflamatórios promovem neurotoxicidade e até morte celular (BLOCK; HONG, 2005).

Uma segunda via de ativação de mecanismos do estresse oxidativo induzido pelo etanol é pelo seu metabolismo específico por enzimas como o citocromo P-450 (CYP) 2E1 e álcool desidrogenase para gerar radicais hidroxietila e acetaldeído, que tem propriedades tóxicas (DEITRICH; ZIMATKIN; PRONKO, 2006; SARC; WRABER; LIPNIK-STANGELJ, 2011). O acetaldeído será posteriormente oxidado a acetato por uma acetaldeído desidrogenase (Figura 1). De fato, o álcool pode aumentar a expressão do CYP2E1 e, assim, a formação de Espécies Reativas de Oxigênio (EROS). Além dessas vias, o estresse oxidativo induzido pelo etanol pode ser gerado por via de ativação direta do complexo enzimático NADPH oxidase da cadeia respiratória mitocondrial (Figura 1). O álcool é metabolizado principalmente no fígado, mas também já foi demonstrado que ocorre este processo no cérebro (HAORAH et al., 2008).

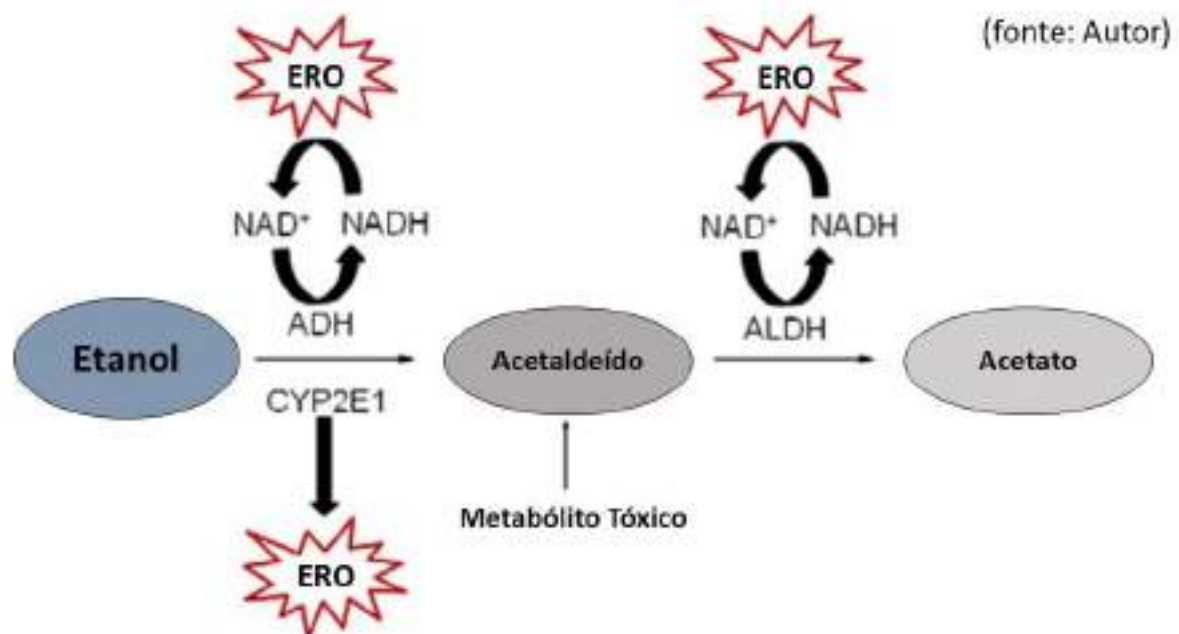


Figura 1. Metabolismo do etanol pelo CYP2E1, álcool desidrogenase (ADH) e acetaldeído desidrogenase (ALDH). Indução de estresse oxidativo

Um aumento de geração de EROS induzido pelo álcool é potencialmente prejudicial. Essas moléculas são capazes de reagir e lesionar constituintes celulares essenciais, como proteínas, lipídios e DNA (WU; CEDERBAUM, 2009), causando anormalidades funcionais e estruturais do cérebro (HARPER, 2009; WANG et al., 2012). Harper (HARPER, 2009) mostrou uma perda neuronal em regiões específicas do córtex cerebral, hipotálamo e cerebelo em alcoólatras, além disso, foi observado um aumento significativo de danos ao DNA nessas regiões em ratos com uso crônico de álcool (RENIS et al., 1996). O excesso de produção de EROS tem sido proposto como uma cause muito importante de dano neuronal ocasionado por etanol (BUSTAMANTE et al., 2012; FOWLER et al., 2014; HEATON et al., 2006; WANG et al., 2012).

O álcool induz dano ao DNA e a falha do reparo destes danos que podem ser precedidos pelo estresse oxidativo especialmente no córtex frontal superior e no hipocampo de alcoolistas (HARPER; MATSUMOTO, 2005). Outros estudos mostram o dano ao DNA pode ser induzido por doses moderadas até muito elevadas de álcool em modelos animais (BUSTAMANTE et al., 2012; DENG; DEITRICH, 2008; GUO et al., 2007; HEATON et al., 2006). Demonstramos que mesmo na abstinência de doses baixas e intermitentes de álcool provocam estas lesões neuronais e dano ao DNA ocasionados pelo aumento do estresse oxidativo em várias regiões do SNC como Cortex pré-frontal, caudato, hipotálamo, hipocampo e cerebelo e em algumas destas regiões houve correlação entre o grau de dano do DNA e a intensidade do estresse oxidativo (COSTA et al., 2015).

1.2 Cafeína

Atualmente a cafeína é a substância psicoativa mais consumida no mundo, principalmente sob a forma de café e bebidas tipos cola, assim como as associações com energéticos, entre outras, havendo consumo médio é de 80 a 400 mg por pessoa/dia. Nesta quantidade, a cafeína alcança níveis plasmáticos de 5 a 20µM. Uma xícara de café contém cerca de 100 mg de cafeína, e uma lata de bebida tipo cola contem até 55 mg de cafeína, enquanto que a concentração de cafeína nas bebidas energéticas pode variar de 50 mg a 500 mg de cafeína por

recipiente dependendo da marca (ARRIA et al., 2011; BRYANT LUDDEN; WOLFSON, 2010; REISSIG; STRAIN; GRIFFITHS, 2009).

Segundo Harnack e cols (HARNACK; STANG; STORY, 1999), em 30 anos, o consumo de cafeína entre adolescentes e crianças aumentou 70% nos Estados Unidos. No Brasil, a quantidade média de cafeína ingerida é de aproximadamente 41 mg (ARRIA et al., 2011; BRYANT LUDDEN; WOLFSON, 2010; REISSIG; STRAIN; GRIFFITHS, 2009). A ingestão de cafeína, mesmo em excesso, é bem aceita socialmente porque as metilxantinas têm propriedades de ativação e preservação de atenção que podem ajudar a produtividade e melhorar o desempenho. As maiores fontes de cafeína para adolescentes e crianças são bebidas energéticas e refrigerantes. Com o surgimento de novos produtos no mercado, adolescentes passam a ter maiores opções de produtos cafeínados, esses produtos incluem cafeína em pó, pílulas de cafeína e principalmente bebidas energéticas (ARRIA et al., 2011; BRYANT LUDDEN; WOLFSON, 2010; REISSIG; STRAIN; GRIFFITHS, 2009). Embora as bebidas energéticas contenham também outros componentes com efeitos psicoativos, como taurina, estudos recentes mostram que a cafeína é o ingrediente ativo responsável pelos efeitos comportamentais e cognitivos associados a essas bebidas (GILES et al., 2012). Em geral, as bebidas energéticas contêm cafeína em concentrações bastante altas. Nos Estados Unidos, 34% dos jovens entre 18 e 24 anos são consumidores de bebidas energéticas (O'BRIEN et al., 2008; WELLS et al., 2013) e entre os estudantes universitários, os percentuais de consumo aumentam para 60% (PRICE et al., 2010). Além disso, essas bebidas são frequentemente consumidas em combinação com outras substâncias que têm potencial de abuso, principalmente com o álcool (MORELLI; SIMOLA, 2011).

1.2.2 Aspectos neurobiológicos e comportamentais da cafeína

A cafeína (1,3,7-trimetilxantina) faz parte do grupo das metilxantinas, grupo que inclui a cafeína, a teofilina e a teobromina. Tais substâncias são normalmente derivadas de plantas. Quimicamente, a cafeína é classificada como um alcalóide purínico e, na farmacologia, como uma substância psicoestimulante (JEFFERY HURST; MARTIN; TARKA, 1997) com seu principal alvo de ação no sistema adenosinérgico, com ação antagonista não seletiva de receptores de adenosina

(DAVIS et al., 2003; FREDHOLM; LINDSTROM, 1999; HIGDON; FREI, 2006). Os efeitos da cafeína no comportamento são mediados pelo bloqueio dos receptores de adenosina A1 e / ou A2A (DALY et al., 1994).

Sendo um psicoestimulante, possui propriedades excitatórias psíquicas e motoras (BRUNTON, 2012), causando sintomas como aumento do estado de alerta, elevação do humor e do foco, redução da fadiga e do apetite (PIERCE; KUMARESAN, 2006). A cafeína, sendo um antagonista do receptor da adenosina, causa uma diminuição da atuação da adenosina. Dada a relação entre níveis elevados de adenosina e sedação / redução da vigilância cognitiva (CHRISTIE et al., 2008; MINGOTE et al., 2008; PORKKA-HEISKANEN et al., 1997), as propriedades psicoestimulantes da cafeína podem ser mediadas pelo bloqueio desses receptores (FREDHOLM et al., 1999, 2005). A maior parte das pesquisas com cafeína geralmente concorda que as propriedades psicoestimulantes da cafeína são, em grande parte, devido ao antagonismo do A₂AR (FERRE et al., 2008). O bloqueio do A₂AR, mas não o A₁R, parece ser um mecanismo importante no desenvolvimento de aumento da atividade locomotora (BROCKWELL; BENINGER, 1996; HSU et al., 2009). Estudos com animais mostram que a cafeína é capaz de melhorar o desempenho em tarefas de memória de trabalho e no labirinto de cruz elevado em função principalmente de seu antagonismo do receptor A₁R (PREDIGER et al., 2005).

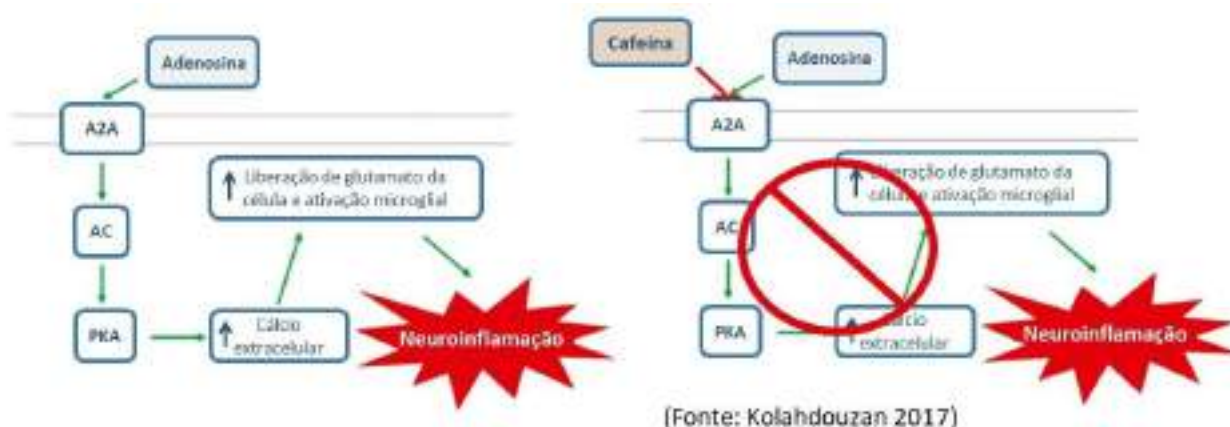
A parada do consumo crônico de cafeína pode produzir sintomas de síndrome de abstinência, cujos sinais e sintomas incluem a diminuição da energia, dificuldade de concentração, diminuição do estado de alerta, cefaleia e cansaço (GRIFFITHS; WOODSON, 1988; JULIANO; GRIFFITHS, 2004). Esses sintomas aparecem entre 12 a 24h de abstinência e podem durar até 6 dias (JULIANO; GRIFFITHS, 2004). Em estudos com animais, foi possível demonstrar que a abstinência de cafeína ocasiona menor tempo de ambulação (NIKODIJEVIĆ; JACOBSON; DALY, 1993). Alguns estudos sugerem que a síndrome de abstinência a cafeína é uma desregulação do sistema adenosinérgico (FREDHOLM et al., 1999; NIKODIJEVIĆ; JACOBSON; DALY, 1993), quando esse sistema é hiperestimulado constantemente pode causar um up-regulation de receptores de adenosina (ESPINOSA et al., 2013; HOLTZMAN; MANTE; MINNEMAN, 1991; PAUL; KURUNWUNE; BIAGGIONI, 1993). Importaneamente, há evidências de uma

interação entre os receptores de adenosina e dopamina (FERRE et al., 1991, 1997; OKADA; MIZUNO; KANEKO, 1996), onde a cafeína seria capaz de aumentar a liberação de dopamina (OKADA; MIZUNO; KANEKO, 1996; SOLINAS et al., 2002).

1.2.3 Cafeína na Neurotoxicidade

Alguns estudos apontam a cafeína tendo uma ação antioxidante interagindo com radicais hidroxila, reduzindo a peroxidação lipídica, aumentando a concentração de glutathiona. Além disso, a cafeína pode aumentar a atividade das enzimas antioxidantes glutathiona redutase e superóxido dismutase (ABREU et al., 2011; NOSCHANG et al., 2009a, 2009b), inibindo o dano oxidativo (SHI; DALAL; JAIN, 1991) e diminuindo o dano no DNA em animais (FERK et al., 2013) ou em humanos (BAKURADZE et al., 2016; LOGANATHAN et al., 2013). Apesar de estudos mostrarem efeitos antioxidantes, esses efeitos podem variar de acordo com as concentrações e quantidades utilizadas. Quando utilizada em doses mais baixas, a cafeína tem efeitos antioxidantes, mas em doses mais altas, tem efeitos pró-oxidante. Em um estudo comparando (TIWARI et al., 2014) duas doses de cafeína em culturas de células epiteliais alveolares expostas à hiperoxia, os níveis de H_2O_2 diminuíram com a dose mais baixa de cafeína (0,05 Mm) e aumentou com exposição a 0,1Mm de cafeína.

A cafeína também expressa seus efeitos neuroprotetores através do antagonismo de receptores A_2A de células gliais (Figura 2), reduzindo a ativação da microglia, inibindo a liberação de citocinas, radicais livres e glutamato (BAGGA; CHUGANI; PATEL, 2016; POSTUMA et al., 2012).



Através da ativação do A₂AR, a adenosina aumenta a ativação da adenilil ciclase (AC), levando à ativação da proteína quinase A (PKA), que causa o aumento do cálcio extracelular dentro da célula, resultando em um aumento na liberação de glutamato da célula, o que leva à neuroinflamação. No entanto, ao inibir a ligação da adenosina ao seu receptor, a cafeína regula negativamente a ativação da PKA, causando um menor aumento do cálcio extracelular no interior da célula, diminuindo a liberação de glutamato da célula, reduzindo assim a neuroinflamação (BAGGA; CHUGANI; PATEL, 2016; KOLAHDOUZAN; HAMADEH, 2017; POSTUMA et al., 2012; SONSALLA et al., 2012).

1.3 Interação Cafeína e Álcool

As bebidas energéticas contêm altas quantidades de cafeína e são frequentemente utilizadas com álcool (MILLER, 2008; REISSIG; STRAIN; GRIFFITHS, 2009). Em um estudo feito pela Autoridade Europeia para a Segurança dos Alimentos (EFSA) sobre o consumo de bebidas energéticas na União Europeia, mostrou que 68% dos adolescentes (10 e 18 anos de idade), utilizavam bebidas energéticas (BREDA et al., 2014). Nos EUA, cerca de 31% dos adolescentes utilizam com regularidade essa combinação (SEPKOWITZ, 2013). No Brasil, não existem dados epidemiológicos atuais sobre essa relação.

Há evidências de um risco elevado de um alto consumo de etanol com cafeína. Isso destaca a importância de compreender os motivos de seu consumo. Alguns estudos mostram que o consumo de álcool misturado com bebidas energéticas está associado ao uso de uma maior quantidade de álcool quando comparado a uso do álcool sozinho (FERREIRA et al., 2006; MARCZINSKI et al., 2016; MARCZINSKI; FILLMORE, 2006). Este efeito pode ser explicado pelo fato da cafeína minimizar os efeitos aversivos do álcool (PEACOCK; BRUNO; MARTIN, 2013; REISSIG; STRAIN; GRIFFITHS, 2009; VELAZQUEZ et al., 2012). Os dados de autorrelatos das populações de jovens e universitários apontam para um maior consumo de álcool (MARMORSTEIN, 2019) e um o antagonismo da sedação induzida pelo etanol quando do consumo associado com cafeína (WELDY, 2010), pelo aumento da estimulação (FERREIRA et al., 2004; MARCZINSKI, 2011) havendo capacidade de beber por mais tempo (ATTWOOD et al., 2012; MARCZINSKI; FILLMORE, 2006) como razões para consumir essas bebidas,

álcool e cafeína, associadas. Outras explicações podem envolver o aumento do desejo pelo consumo de álcool induzido por energéticos. Um estudo recente (DROSTE et al., 2014) apontou que os principais motivos para o uso concomitante de álcool com bebidas cafeínadas, citados pelos indivíduos da pesquisa foram: motivos hedonístico (consumir bebidas alcoólicas com cafeína é mais prazeroso), social (facilitação social), vigor/dinamismo (mais tempo para beber e festa) e redução da percepção da intoxicação. Dentre estes motivos, os hedonistas e menor percepção de intoxicação foram associados ao aumento do risco de maior consumo dessas bebidas. Neste sentido, o uso de bebidas energéticas com álcool tem sido associado a uma propensão maior para a dependência do álcool, a longo prazo (ARRIA et al., 2011; CHENG et al., 2012).

Há também dados de que o comprometimento comportamental não mostra mudanças significativas nas sensações subjetivas de inibição da atividade do SNC e sedação, ansiedade, embriaguez ou intoxicação (ARRIA et al., 2011; PEACOCK; BRUNO; MARTIN, 2013). Tem sido sugerido que, com a adição de cafeína ao álcool, a mudança qualitativa na intoxicação se deve principalmente a um aumento na estimulação autorreferida, embora não necessariamente haja uma redução quantitativa na intoxicação por si só (ATTWOOD et al., 2012). A cafeína atenua as mudanças induzidas pelo etanol em parâmetros psicológicos, como processamento de informações, memória e desempenho psicomotor (FERRE; O'BRIEN, 2011). Em contraste, a combinação de álcool e cafeína não produziu impacto no tempo de reação em comparação com o grupo de álcool sozinho (AZCONA et al., 1995). Além disso, em tarefas de controle inibitório, como sinalizações sem movimento ou com sinal de parada, os resultados mostram resultados mistos (FERRE; O'BRIEN, 2011). Também, o comprometimento da função cognitiva e motora relacionado ao álcool parece não ser afetado pelo consumo de cafeína (ULBRICH et al., 2013; WELDY, 2010).

Assim, a cafeína melhora o prejuízo relacionado ao uso de álcool para algumas tarefas, enquanto que em outras não produz nenhum efeito (ATTWOOD et al., 2012; MARCZINSKI; FILLMORE, 2003). Por exemplo, uma concentração de cafeína de 80 mg administrada em uma bebida energética pode não ser suficiente para antagonizar todos os efeitos das diferentes doses de álcool (0,6 e 1,0 g / kg) (FERREIRA et al., 2004). Embora nessa combinação de doses possa haver

redução da sensação subjetiva de intoxicação com diminuição da cefaleia, redução da sensação de fadiga, menos tontura, menos problemas ao caminhar ou menos boca seca (FERREIRA et al., 2004, 2006). Por outro lado, não foram alterados parâmetros de teste de motricidade visual, tempo de performance e reação, parâmetros fisiológicos de coordenação e medidas bioquímicas e comportamentais avaliadas objetivamente, em humanos intoxicados com álcool ou intoxicados com álcool mais uso de cafeínados (FERREIRA et al., 2004, 2006). Em outro estudo, uma maior concentração de cafeína (400 mg) e uma mais baixa dose de álcool (0,6 g / kg) melhorou o desempenho psicomotor em relação aos indivíduos que consumiam apenas álcool. No entanto, não houve melhora em parâmetros como a capacidade de dirigir um carro em um simulador (LIGUORI; ROBINSON, 2001). Portanto, inúmeros estudos apontam que a sensação subjetiva de intoxicação alcoólica é reduzida pela cafeína, mas não a intoxicação em si (RIESSELMANN; ROSENBAUM; SCHNEIDER, 1996).

Estudos em animais têm sido empregados para entender melhor os efeitos da cafeína no consumo de álcool. Até agora, os resultados são variados com alguns estudos que demonstram um aumento na ingestão de etanol a partir do efeito cafeína (KUNIN et al., 2000; REZVANI et al., 2013) e outros estudos que não demonstram efeito da cafeína (FRITZ; COMPANION; BOEHM, 2014; HUGHES, 2011), e há, também, estudos mostrando uma diminuição na ingestão de etanol induzida pela cafeína (REZVANI et al., 2013). A administração concomitante de cafeína (5-40 mg / kg) não teve efeito sobre as diminuições na aprendizagem induzidas pelo álcool, na tarefa de discriminar a evitação do labirinto de cruz elevada (ansiedade) (GULICK; GOULD, 2009) ou no teste de reconhecimento de objetos (FRITZ; COMPANION; BOEHM, 2014). No entanto, observa-se que baixa dose cafeína (5 mg / kg) reduz a perda de memória retrógrada induzida pelo álcool em ratos (SPINETTA et al., 2008). Em animais, a cafeína reduz a sedação induzida pelo álcool, deixando os animais mais estimulados, menos atáxicos e menos ansiosos (FRITZ; COMPANION; BOEHM, 2014). A maioria dos estudos com roedores sobre a coexposição de cafeína e álcool usou a administração forçada, por injeções sistêmicas ou gavagens intragástricas das duas drogas (EL YACOUBI et al., 2003; FERREIRA et al., 2004; GULICK; GOULD, 2009; KUNIN et al., 2000; REZVANI et al., 2013; SPINETTA et al., 2008). É bem conhecido que as

administrações forçadas podem produzir mudanças significativas no comportamento e na neurobiologia (JACOBS et al., 2003; MITCHELL et al., 2012; MOOLTEN; KORNETSKY, 1990). Portanto, é extremamente relevante desenvolver estudos com os modelos animais de autoadministração que têm maior validade translacional em relação à experiência humana.

Há muito poucos estudos em animais com foco no impacto do uso da cafeína na abstinência de álcool. No entanto, há evidências de que outros moduladores dos receptores de adenosina, tanto agonistas e quanto antagonistas, regulam alguns sinais de abstinência de álcool. A administração de um agonista seletivo do receptor A1 de adenosina, o2-cloro-N6-ciclopentiladenosina (CCPA), após uma injeção aguda de etanol em camundongos foi capaz de reduzir a ansiedade observada em um labirinto em cruz elevado. Por outro lado, o agonista seletivo do receptor A2A da adenosina, N6-[2-(3,5-dimetoxifenil)-2-(2-metilfenil)-etil]adenosina, não teve o mesmo efeito (PREDIGER et al., 2006). Também foi descrito que antagonismo dos receptores A1 durante a ingestão crônica de álcool aumenta o tempo de desenvolvimento de tolerância ao álcool em ratos (BATISTA et al., 2005) e o bloqueio de receptores A2A diminuiu convulsões causadas pela abstinência de álcool (EL YACOUBI et al., 2001). Levando em conta estes estudos, foi concluído que o antagonismo dos receptores A1 e / ou A2A da adenosina pela cafeína durante o consumo de álcool poderia ter um papel na prevenção da abstinência durante a ingestão de álcool (EL YACOUBI et al., 2001; GATCH; SELVIG, 2002).

2. JUSTIFICATIVA

O abuso de álcool é um problema de saúde mundial, com graves consequências médicas, econômicas e sociais. Os efeitos da exposição crônica ao álcool e sua retirada são amplamente estudados e, ainda assim, não estão totalmente elucidados. No entanto, é aceito que a exposição crônica ao etanol produz mudanças adaptativas na função, afinidade e densidade de múltiplas proteínas receptoras cerebrais. Essas mudanças tornam o cérebro vulnerável a insultos excitotóxicos durante os ciclos de consumo e também de abstinência do álcool (COSTA et al., 2015; DE WITTE, 2004; KOOB, 2003; KOOB et al., 2004; PRENDERGAST et al., 2004).

Estudos mostram que o consumo de etanol misturado com bebidas energéticas, que tem como principal componente a cafeína, está associado ao uso de uma maior quantidade de álcool quando comparado ao seu uso isolado (FERREIRA et al., 2006; MARCZINSKI et al., 2016; MARCZINSKI; FILLMORE, 2006). Neste sentido, o uso de álcool em associação à bebidas energéticas tem sido relacionado a uma propensão de maior consumo, elevando, conseqüentemente, as chances de desenvolvimento de dependência do álcool a longo prazo (ARRIA et al., 2011; CHENG et al., 2012). Além da dependência, uso de álcool tem sido uma preocupação importante em função do aumento da produção de fatores pró-inflamatórios e neurotóxicos ocasionados por seu consumo (WU; CEDERBAUM, 2009). Já a cafeína, quando utilizada em doses elevadas, também pode exercer efeitos pró-oxidantes, mas, em doses mais baixas, tem efeitos antioxidantes (TIWARI et al., 2014).

Em estudo anterior, descrevemos manifestações comportamentais de abstinência de álcool e as lesões de DNA associadas no SNC em um modelo com roedores (COSTA et al., 2015). Percebemos que a busca de conhecimentos sobre substâncias que podem interferir com as manifestações de consumo e abstinência de doses variadas de álcool são necessárias. Neste sentido, é importante estudar as relações, tanto comportamentais, relativas aqui à síndrome de abstinência, quanto neurotóxicas, da associação álcool e cafeína. Ainda que extensivamente praticado, o uso concomitante de álcool e bebidas contendo cafeína, ainda necessita de respostas acerca dos efeitos novos e desconhecidos que podem surgir desta interação.

3. OBJETIVOS

3.1 Objetivo Geral

Avaliar os comportamentos na autoadministração de álcool e cafeína, as manifestações tipo depressivas quando em abstinência, com as substâncias apresentadas isoladamente ou concomitantemente, e o dano de DNA no cérebro de ratos machos púberes.

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3.2 Objetivos Específicos

Determinar se há diferenças de consumo entre animais expostos ao álcool associado ou não com cafeína.

Avaliar se a presença de cafeína modifica comportamentos induzidos pelo álcool no teste do nado forçado em ratos com abstinência de 24 horas.

Avaliar se o consumo continuado de cafeína modifica comportamentos induzidos pelo álcool.

Avaliar os efeitos da autoadministração do álcool e da cafeína sobre o dano de DNA em células do córtex pré-frontal dos ratos machos por meio ensaio cometa.

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4. REFERÊNCIAS BIBLIOGRÁFICAS

ABREU, R. V. et al. Chronic coffee and caffeine ingestion effects on the cognitive function and antioxidant system of rat brains. **Pharmacology, biochemistry, and behavior**, v. 99, n. 4, p. 659–664, out. 2011.

ALFONSO-LOECHES, S. et al. Pivotal role of TLR4 receptors in alcohol-induced neuroinflammation and brain damage. **The Journal of neuroscience : the official journal of the Society for Neuroscience**, v. 30, n. 24, p. 8285–8295, jun. 2010.

ALLEN-GIPSON, D. S. et al. Ethanol blocks adenosine uptake via inhibiting the nucleoside transport system in bronchial epithelial cells. **Alcoholism, clinical and experimental research**, v. 33, n. 5, p. 791–798, maio 2009.

ARRIA, A. M. et al. Energy Drink Consumption and Increased Risk for Alcohol Dependence. **Alcoholism: Clinical and Experimental Research**, v. 35, n. 2, p. 365–375, 2011.

ATTWOOD, A. S. et al. Effects of caffeine on alcohol-related changes in behavioural control and perceived intoxication in light caffeine consumers. **Psychopharmacology**, v. 221, n. 4, p. 551–560, jun. 2012.

AZCONA, O. et al. Evaluation of the central effects of alcohol and caffeine interaction. **British journal of clinical pharmacology**, v. 40, n. 4, p. 393–400, out. 1995.

BAGGA, P.; CHUGANI, A. N.; PATEL, A. B. Neuroprotective effects of caffeine in MPTP model of Parkinson's disease: A (13)C NMR study. **Neurochemistry international**, v. 92, p. 25–34, jan. 2016.

BAKURADZE, T. et al. Coffee consumption rapidly reduces background DNA strand breaks in healthy humans: Results of a short-term repeated uptake intervention study. **Molecular nutrition & food research**, v. 60, n. 3, p. 682–686, mar. 2016.

BALDWIN, H. A. et al. CRF antagonist reverses the “anxiogenic” response to ethanol withdrawal in the rat. **Psychopharmacology**, v. 103, n. 2, p. 227–232, 1991.

BATISTA, L. C. et al. Blockade of adenosine and dopamine receptors inhibits the development of rapid tolerance to ethanol in mice. **Psychopharmacology**, v. 181, n. 4, p. 714–721, 2005.

BEYER, M. et al. Phagocytosis of neuronal or glial debris by microglial cells: upregulation of MHC class II expression and multinuclear giant cell formation in vitro. **Glia**, v. 31, n. 3, p. 262–266, set. 2000.

BLOCK, M. L.; HONG, J.-S. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. **Progress in neurobiology**, v. 76, n. 2, p. 77–98, jun. 2005.

BONASSOLI, V. T.; MILANI, H.; DE OLIVEIRA, R. M. W. Ethanol withdrawal activates nitric oxide-producing neurons in anxiety-related brain areas. **Alcohol**

(Fayetteville, N.Y.), v. 45, n. 7, p. 641–652, nov. 2011.

BRANDES, S. M. Clinical Handbook of Psychotropic Drugs, 20th ed ., by K. Z. Bezchlibnyk-Butler, J. J. Jeffries, R. M. Procyshyn, and A. S. Virani. **Journal of Community Health Nursing**, v. 31, n. 2, p. 130–130, 3 abr. 2014.

BREDA, J. J. et al. Energy drink consumption in europe: a review of the risks, adverse health effects, and policy options to respond. **Frontiers in public health**, v. 2, p. 134, 2014.

BROCKWELL, N. T.; BENINGER, R. J. The differential role of A1 and A2 adenosine receptor subtypes in locomotor activity and place conditioning in rats. **Behavioural pharmacology**, v. 7, n. 4, p. 373–383, ago. 1996.

BRUNTON, L. L. **As bases farmacológicas da terapêutica de Goodman & Gilman**. [s.l: s.n.].

BRYANT LUDDEN, A.; WOLFSON, A. R. Understanding adolescent caffeine use: connecting use patterns with expectancies, reasons, and sleep. **Health education & behavior : the official publication of the Society for Public Health Education**, v. 37, n. 3, p. 330–342, jun. 2010.

BUSTAMANTE, J. et al. Alterations of motor performance and brain cortex mitochondrial function during ethanol hangover. **Alcohol (Fayetteville, N.Y.)**, v. 46, n. 5, p. 473–479, ago. 2012.

CABRAL, A. et al. Fear state induced by ethanol withdrawal may be due to the sensitization of the neural substrates of aversion in the dPAG. **Experimental neurology**, v. 200, n. 1, p. 200–208, jul. 2006.

CHENG, W. J. et al. Alcohol dependence, consumption of alcoholic energy drinks and associated work characteristics in the Taiwan working population. **Alcohol and Alcoholism**, v. 47, n. 4, p. 372–379, 2012.

CHOI, D.-S. et al. The type 1 equilibrative nucleoside transporter regulates ethanol intoxication and preference. **Nature neuroscience**, v. 7, n. 8, p. 855–861, ago. 2004.

CHRISTIE, M. A. et al. Microdialysis elevation of adenosine in the basal forebrain produces vigilance impairments in the rat psychomotor vigilance task. **Sleep**, v. 31, n. 10, p. 1393–1398, out. 2008.

COLOMBO, G. et al. Role of GABA(B) receptor in alcohol dependence: reducing effect of baclofen on alcohol intake and alcohol motivational properties in rats and amelioration of alcohol withdrawal syndrome and alcohol craving in human alcoholics. **Neurotoxicity research**, v. 6, n. 5, p. 403–414, 2004.

COSTA, P. A. et al. Brain DNA damage and behavioral changes after repeated intermittent acute ethanol withdrawal by young rats. **Psychopharmacology**, v. 232, n. 19, p. 3623–3636, 2015.

DALY, J. W. et al. The role of adenosine receptors in the central action of caffeine. **Pharmacopschoecologia**, v. 7, n. 2, p. 201–213, 1994.

DAVIS, J. M. et al. Central nervous system effects of caffeine and adenosine on fatigue. **American journal of physiology. Regulatory, integrative and comparative physiology**, v. 284, n. 2, p. R399-404, fev. 2003.

DE WITTE, P. Imbalance between neuroexcitatory and neuroinhibitory amino acids causes craving for ethanol. **Addictive behaviors**, v. 29, n. 7, p. 1325–1339, set. 2004.

DEITRICH, R.; ZIMATKIN, S.; PRONKO, S. Oxidation of ethanol in the brain and its consequences. **Alcohol research & health : the journal of the National Institute on Alcohol Abuse and Alcoholism**, v. 29, n. 4, p. 266–273, 2006.

DENG, X.; DEITRICH, R. A. Putative role of brain acetaldehyde in ethanol addiction. **Current drug abuse reviews**, v. 1, n. 1, p. 3–8, jan. 2008.

DOREMUS-FITZWATER, T. L.; SPEAR, L. P. Developmental differences in acute ethanol withdrawal in adolescent and adult rats. **Alcoholism, clinical and experimental research**, v. 31, n. 9, p. 1516–1527, set. 2007.

DROSTE, N. et al. Combined alcohol and energy drink use: motivations as predictors of consumption patterns, risk of alcohol dependence, and experience of injury and aggression. **Alcoholism, clinical and experimental research**, v. 38, n. 7, p. 2087–2095, jul. 2014.

EL YACOUBI, M. et al. Absence of the adenosine A(2A) receptor or its chronic blockade decrease ethanol withdrawal-induced seizures in mice. **Neuropharmacology**, v. 40, n. 3, p. 424–432, mar. 2001.

EL YACOUBI, M. et al. Caffeine reduces hypnotic effects of alcohol through adenosine A2A receptor blockade. **Neuropharmacology**, v. 45, n. 7, p. 977–985, dez. 2003.

ESPINOSA, J. et al. Caffeine consumption prevents memory impairment, neuronal damage, and adenosine A2A receptors upregulation in the hippocampus of a rat model of sporadic dementia. **Journal of Alzheimer's Disease**, v. 34, n. 2, p. 509–518, 2013.

FERK, F. et al. Protective effects of coffee against induction of DNA damage and pre-neoplastic foci by aflatoxin B 1. p. 1–10, 2013.

FERRE, S. et al. Stimulation of high-affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. **Proceedings of the National Academy of Sciences of the United States of America**, v. 88, n. 16, p. 7238–7241, ago. 1991.

FERRE, S. et al. Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. **Trends in neurosciences**, v. 20, n. 10, p. 482–487, out. 1997.

FERRE, S. et al. Adenosine A1-A2A receptor heteromers: new targets for caffeine in the brain. **Frontiers in bioscience : a journal and virtual library**, v. 13, p. 2391–2399, jan. 2008.

FERRE, S.; O'BRIEN, M. C. **Alcohol and Caffeine: The Perfect Storm.**Journal of caffeine researchUnited States, set. 2011.

FERREIRA, S. E. et al. Can energy drinks reduce the depressor effect of ethanol? An experimental study in mice. **Physiology & behavior**, v. 82, n. 5, p. 841–847, out. 2004.

FERREIRA, S. E. et al. Effects of energy drink ingestion on alcohol intoxication. **Alcoholism, clinical and experimental research**, v. 30, n. 4, p. 598–605, abr. 2006.

FISCHER, W.; FRANKE, H.; ILLES, P. Effects of acute ethanol on the Ca²⁺ response to AMPA in cultured rat cortical GABAergic nonpyramidal neurons. **Alcohol and alcoholism (Oxford, Oxfordshire)**, v. 38, n. 5, p. 394–399, 2003.

FOWLER, A.-K. et al. Differential sensitivity of prefrontal cortex and hippocampus to alcohol-induced toxicity. **PloS one**, v. 9, n. 9, p. e106945, 2014.

FREDHOLM, B. B. et al. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. **Pharmacological reviews**, v. 51, n. 1, p. 83–133, mar. 1999.

FREDHOLM, B. B. et al. Actions of adenosine at its receptors in the CNS: insights from knockouts and drugs. **Annual review of pharmacology and toxicology**, v. 45, p. 385–412, 2005.

FREDHOLM, B. B.; LINDSTROM, K. Autoradiographic comparison of the potency of several structurally unrelated adenosine receptor antagonists at adenosine A₁ and A_{2A} receptors. **European journal of pharmacology**, v. 380, n. 2–3, p. 197–202, set. 1999.

FRITZ, B. M.; COMPANION, M.; BOEHM, S. L. “Wired,” Yet Intoxicated: Modeling Binge Caffeine and Alcohol Co-Consumption in the Mouse. **Alcoholism: Clinical and Experimental Research**, v. 38, n. 8, p. 2269–2278, 2014.

GATCH, M. B.; SELVIG, M. Theophylline blocks ethanol withdrawal-induced hyperalgesia. **Alcohol and alcoholism (Oxford, Oxfordshire)**, v. 37, n. 4, p. 313–317, 2002.

GIGLIOTTI, A.; BESSA, M. A. **Síndrome de Dependência do Álcool: Critérios diagnósticos**Revista Brasileira de Psiquiatria, 2004.

GILES, G. E. et al. Differential cognitive effects of energy drink ingredients: caffeine, taurine, and glucose. **Pharmacology, biochemistry, and behavior**, v. 102, n. 4, p. 569–577, out. 2012.

GINSBURG, B. C.; LAMB, R. J. Reinforcement of an alternative behavior as a model of recovery and relapse in the rat. **Behavioural processes**, v. 94, p. 60–66, mar. 2013.

GRANT, B. F. The impact of a family history of alcoholism on the relationship between age at onset of alcohol use and DSM-IV alcohol dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. **Alcohol health and**

research world, v. 22, n. 2, p. 144–147, 1998.

GRIFFITHS, R. R.; WOODSON, P. P. Caffeine physical dependence: a review of human and laboratory animal studies. **Psychopharmacology**, v. 94, n. 4, p. 437–451, 1988.

GULICK, D.; GOULD, T. J. Effects of Ethanol and Caffeine on Behavior in C57BL/6 Mice in the Plus-Maze Discriminative Avoidance Task. **Behavioral Neuroscience**, v. 123, n. 6, p. 1271–1278, 2009.

GUO, L. et al. Direct in vivo evidence of protective effects of grape seed procyanidin fractions and other antioxidants against ethanol-induced oxidative DNA damage in mouse brain cells. **Journal of agricultural and food chemistry**, v. 55, n. 14, p. 5881–5891, jul. 2007.

HAORAH, J. et al. Mechanism of alcohol-induced oxidative stress and neuronal injury. **Free radical biology & medicine**, v. 45, n. 11, p. 1542–1550, dez. 2008.

HARNACK, L.; STANG, J.; STORY, M. Soft drink consumption among US children and adolescents: nutritional consequences. **Journal of the American Dietetic Association**, v. 99, n. 4, p. 436–441, abr. 1999.

HARPER, C. The neuropathology of alcohol-related brain damage. **Alcohol and alcoholism (Oxford, Oxfordshire)**, v. 44, n. 2, p. 136–140, 2009.

HARPER, C.; MATSUMOTO, I. **Ethanol and brain damage** *Current Opinion in Pharmacology*, 2005.

HEATON, M. B. et al. Effect of bax deletion on ethanol sensitivity in the neonatal rat cerebellum. **Journal of neurobiology**, v. 66, n. 1, p. 95–101, jan. 2006.

HIGDON, J. V; FREI, B. Coffee and health: a review of recent human research. **Critical reviews in food science and nutrition**, v. 46, n. 2, p. 101–123, 2006.

HOLTZMAN, S. G.; MANTE, S.; MINNEMAN, K. P. Role of adenosine receptors in caffeine tolerance. **The Journal of pharmacology and experimental therapeutics**, v. 256, n. 1, p. 62–68, jan. 1991.

HSU, C. W. et al. Caffeine and a selective adenosine A2A receptor antagonist induce reward and sensitization behavior associated with increased phospho-Thr75-DARPP-32 in mice. **Psychopharmacology**, v. 204, n. 2, p. 313–325, jun. 2009.

HUGHES, R. N. Adult anxiety-related behavior of rats following consumption during late adolescence of alcohol alone and in combination with caffeine. **Alcohol (Fayetteville, N.Y.)**, v. 45, n. 4, p. 365–372, jun. 2011.

JACOBS, E. H. et al. Neuroadaptive effects of active versus passive drug administration in addiction research. **Trends in pharmacological sciences**, v. 24, n. 11, p. 566–573, nov. 2003.

JEFFERY HURST; MARTIN, R.; TARKA, S. Analytical Methods for Quantitation of Methylxanthines. In: **Caffeine**. [s.l.: s.n.].

JULIANO, R. L. M.; GRIFFITHS, R. R. A critical review of caffeine withdrawal : empirical validation of symptoms and signs , incidence , severity , and associated features. p. 1–29, 2004.

JULIEN, R.; ADVOCAT, C.; COMATY, J. **A primer of drug action (12th ed.)**. [s.l: s.n.].

KOLAHDOUZAN, M.; HAMADEH, M. J. **The neuroprotective effects of caffeine in neurodegenerative diseases****CNS Neuroscience and Therapeutics**, 2017.

KOOB, G. F. Alcoholism: allostasis and beyond. **Alcoholism, clinical and experimental research**, v. 27, n. 2, p. 232–243, fev. 2003.

KOOB, G. F. et al. Neurobiological mechanisms in the transition from drug use to drug dependence. **Neuroscience and biobehavioral reviews**, v. 27, n. 8, p. 739–749, jan. 2004.

KREUTZBERG, G. W. Microglia: a sensor for pathological events in the CNS. **Trends in neurosciences**, v. 19, n. 8, p. 312–318, ago. 1996.

KUNIN, D. et al. Caffeine promotes ethanol drinking in rats. Examination using a limited-access free choice paradigm. **Alcohol (Fayetteville, N.Y.)**, v. 21, n. 3, p. 271–277, jul. 2000.

LIGUORI, A.; ROBINSON, J. H. Caffeine antagonism of alcohol-induced driving impairment. **Drug and alcohol dependence**, v. 63, n. 2, p. 123–129, jul. 2001.

LOGANATHAN, C. et al. Pharmacophore Design, Virtual Screening, Molecular Docking and Optimization Approaches to Discover Potent Thrombin Inhibitors. **Combinatorial Chemistry & High Throughput Screening**, v. 16, n. 9, p. 702–720, 2013.

LOPEZ, M. F.; BECKER, H. C. Operant ethanol self-administration in ethanol dependent mice. **Alcohol**, v. 48, n. 3, p. 295–299, 2014.

MARCZINSKI, C. A. Alcohol mixed with energy drinks: consumption patterns and motivations for use in U.S. college students. **International journal of environmental research and public health**, v. 8, n. 8, p. 3232–3245, ago. 2011.

MARCZINSKI, C. A. et al. Desire to Drink Alcohol is Enhanced with High Caffeine Energy Drink Mixers. **Alcoholism: Clinical and Experimental Research**, v. 40, n. 9, p. 1982–1990, 2016.

MARCZINSKI, C. A.; FILLMORE, M. T. Dissociative antagonistic effects of caffeine on alcohol-induced impairment of behavioral control. **Experimental and clinical psychopharmacology**, v. 11, n. 3, p. 228–236, ago. 2003.

MARCZINSKI, C. A.; FILLMORE, M. T. Clubgoers and their trendy cocktails: implications of mixing caffeine into alcohol on information processing and subjective reports of intoxication. **Experimental and clinical psychopharmacology**, v. 14, n. 4, p. 450–458, nov. 2006.

MARMORSTEIN, N. R. Investigating associations between caffeinated beverage

consumption and later alcohol consumption among early adolescents. **Addictive behaviors**, v. 90, p. 362–368, mar. 2019.

MCKINNEY, A.; COYLE, K. Alcohol hangover effects on measures of affect the morning after a normal night's drinking. **Alcohol and alcoholism (Oxford, Oxfordshire)**, v. 41, n. 1, p. 54–60, 2006.

MILLER, K. E. Energy Drinks, Race, and Problem Behaviors Among College Students. **Journal of Adolescent Health**, v. 43, n. 5, p. 490–497, 2008.

MINGOTE, S. et al. Systemic administration of the adenosine A(2A) agonist CGS 21680 induces sedation at doses that suppress lever pressing and food intake. **Pharmacology, biochemistry, and behavior**, v. 89, n. 3, p. 345–351, maio 2008.

MITCHELL, J. M. et al. Alcohol self-administration, anxiety, and cortisol levels predict changes in delta opioid receptor function in the ventral tegmental area. **Behavioral neuroscience**, v. 126, n. 4, p. 515–522, ago. 2012.

MOOLTEN, M.; KORNETSKY, C. Oral self-administration of ethanol and not experimenter-administered ethanol facilitates rewarding electrical brain stimulation. **Alcohol**, v. 7, n. 3, p. 221–225, 1990.

MORELLI, M.; SIMOLA, N. Methylxanthines and drug dependence: a focus on interactions with substances of abuse. **Handbook of experimental pharmacology**, n. 200, p. 483–507, 2011.

MUKHERJEE, S. et al. Consequences of alcohol consumption on neurotransmitters -an overview. **Current neurovascular research**, v. 5, n. 4, p. 266–272, nov. 2008.

NAGY, L. E. et al. Ethanol increases extracellular adenosine by inhibiting adenosine uptake via the nucleoside transporter. **The Journal of biological chemistry**, v. 265, n. 4, p. 1946–1951, fev. 1990.

NIKODIJEVIĆ, O.; JACOBSON, K. A.; DALY, J. W. Locomotor activity in mice during chronic treatment with caffeine and withdrawal. **Pharmacology, Biochemistry and Behavior**, v. 44, n. 1, p. 199–216, 1993.

NOSCHANG, C. G. et al. Sex-specific differences on caffeine consumption and chronic stress-induced anxiety-like behavior and DNA breaks in the hippocampus. **Pharmacology Biochemistry and Behavior**, v. 94, n. 1, p. 63–69, 2009a.

NOSCHANG, C. G. et al. Interactions between chronic stress and chronic consumption of caffeine on the enzymatic antioxidant system. **Neurochemical research**, v. 34, n. 9, p. 1568–1574, set. 2009b.

O'BRIEN, M. C. et al. Caffeinated cocktails: energy drink consumption, high-risk drinking, and alcohol-related consequences among college students. **Academic emergency medicine : official journal of the Society for Academic Emergency Medicine**, v. 15, n. 5, p. 453–460, maio 2008.

O'CALLAGHAN, J. P.; SRIRAM, K.; MILLER, D. B. Defining "neuroinflammation". **Annals of the New York Academy of Sciences**, v. 1139, p. 318–330, out. 2008.

OKADA, M.; MIZUNO, K.; KANEKO, S. Adenosine A1 and A2 receptors modulate extracellular dopamine levels in rat striatum. **Neuroscience letters**, v. 212, n. 1, p. 53–56, jul. 1996.

PAUL, S.; KURUNWUNE, B.; BIAGGIONI, I. Caffeine withdrawal: apparent heterologous sensitization to adenosine and prostacyclin actions in human platelets. **The Journal of pharmacology and experimental therapeutics**, v. 267, n. 2, p. 838–843, nov. 1993.

PEACOCK, A.; BRUNO, R.; MARTIN, F. H. Patterns of use and motivations for consuming alcohol mixed with energy drinks. **Psychology of addictive behaviors : journal of the Society of Psychologists in Addictive Behaviors**, v. 27, n. 1, p. 202–6, 2013.

PENNING, R. et al. Measurement of alcohol hangover severity: development of the Alcohol Hangover Severity Scale (AHSS). **Psychopharmacology**, v. 225, n. 4, p. 803–810, fev. 2013.

PIERCE, R. C.; KUMARESAN, V. The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? **Neuroscience and biobehavioral reviews**, v. 30, n. 2, p. 215–238, 2006.

PORKKA-HEISKANEN, T. et al. Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness. **Science (New York, N.Y.)**, v. 276, n. 5316, p. 1265–1268, maio 1997.

POSTUMA, R. B. et al. Caffeine for treatment of Parkinson disease: a randomized controlled trial. **Neurology**, v. 79, n. 7, p. 651–658, ago. 2012.

PREDIGER, R. D. S. et al. Caffeine improves spatial learning deficits in an animal model of attention deficit hyperactivity disorder (ADHD) -- the spontaneously hypertensive rat (SHR). **The international journal of neuropsychopharmacology**, v. 8, n. 4, p. 583–594, dez. 2005.

PREDIGER, R. D. S. et al. Activation of adenosine A1 receptors reduces anxiety-like behavior during acute ethanol withdrawal (hangover) in mice. **Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology**, v. 31, n. 10, p. 2210–2220, out. 2006.

PRENDERGAST, M. A. et al. Hippocampal CA1 region neurodegeneration produced by ethanol withdrawal requires activation of intrinsic polysynaptic hippocampal pathways and function of N-methyl-D-aspartate receptors. **Neuroscience**, v. 124, n. 4, p. 869–877, 2004.

PRICE, S. R. et al. Energy drink co-administration is associated with increased reported alcohol ingestion. **Drug and alcohol review**, v. 29, n. 3, p. 331–333, maio 2010.

REISSIG, C. J.; STRAIN, E. C.; GRIFFITHS, R. R. Caffeinated energy drinks-A growing problem. **Drug and Alcohol Dependence**, v. 99, n. 1–3, p. 1–10, 2009.

RENIS, M. et al. Nuclear DNA strand breaks during ethanol-induced oxidative stress in rat brain. **FEBS letters**, v. 390, n. 2, p. 153–156, jul. 1996.

REZVANI, A. H. et al. Effects of caffeine on alcohol consumption and nicotine self-administration in rats. **Alcoholism: Clinical and Experimental Research**, v. 37, n. 9, p. 1609–1617, 2013.

RIESSELMANN, B.; ROSENBAUM, F.; SCHNEIDER, V. [Alcohol and energy drink-can combined consumption of both beverages modify automobile driving fitness?]. **Blutalkohol**, v. 33, n. 4, p. 201–208, jul. 1996.

ROSSETTI, Z. L.; CARBONI, S.; FADDA, F. Glutamate-induced increase of extracellular glutamate through N-methyl-D-aspartate receptors in ethanol withdrawal. **Neuroscience**, v. 93, n. 3, p. 1135–1140, 1999.

SARC, L.; WRABER, B.; LIPNIK-STANGELJ, M. Ethanol and acetaldehyde disturb TNF-alpha and IL-6 production in cultured astrocytes. **Human & experimental toxicology**, v. 30, n. 9, p. 1256–1265, set. 2011.

SEPKOWITZ, K. A. Energy drinks and caffeine-related adverse effects. **JAMA**, v. 309, n. 3, p. 243–244, jan. 2013.

SHI, X.; DALAL, N. S.; JAIN, A. C. Antioxidant behaviour of caffeine: efficient scavenging of hydroxyl radicals. **Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association**, v. 29, n. 1, p. 1–6, jan. 1991.

SOLINAS, M. et al. Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. **The Journal of neuroscience : the official journal of the Society for Neuroscience**, v. 22, n. 15, p. 6321–6324, ago. 2002.

SONSALLA, P. K. et al. Delayed caffeine treatment prevents nigral dopamine neuron loss in a progressive rat model of Parkinson's disease. **Experimental neurology**, v. 234, n. 2, p. 482–487, abr. 2012.

SPANAGEL, R.; KIEFER, F. Drugs for relapse prevention of alcoholism: ten years of progress. **Trends in pharmacological sciences**, v. 29, n. 3, p. 109–115, mar. 2008.

SPINETTA, M. J. et al. Alcohol-induced retrograde memory impairment in rats: prevention by caffeine. **Psychopharmacology**, v. 201, n. 3, p. 361–371, dez. 2008.

TATEBAYASHI, H.; MOTOMURA, H.; NARAHASHI, T. Alcohol modulation of single GABA(A) receptor-channel kinetics. **Neuroreport**, v. 9, n. 8, p. 1769–1775, jun. 1998.

TIWARI, K. K. et al. Differential concentration-specific effects of caffeine on cell viability, oxidative stress, and cell cycle in pulmonary oxygen toxicity in vitro. **Biochemical and biophysical research communications**, v. 450, n. 4, p. 1345–1350, ago. 2014.

ULBRICH, A. et al. Effects of alcohol mixed with energy drink and alcohol alone on subjective intoxication. **Amino acids**, v. 45, n. 6, p. 1385–1393, dez. 2013.

UNODC. World Drug Report 2018: Global overview of drug demand and supply. In: **World Drug Report 2017**. [s.l.: s.n.].

VELAZQUEZ, C. E. et al. Associations between energy drink consumption and alcohol use behaviors among college students. **Drug and Alcohol Dependence**, v. 123, n. 1–3, p. 167–172, 2012.

VENGELIENE, V. et al. **Neuropharmacology of alcohol addiction** **British Journal of Pharmacology**, 2008.

WAKITA, M. et al. Effects of ethanol on GABA(A) receptors in GABAergic and glutamatergic presynaptic nerve terminals. **The Journal of pharmacology and experimental therapeutics**, v. 341, n. 3, p. 809–819, jun. 2012.

WANG, X. et al. Cdc42-Dependent Activation of NADPH Oxidase Is Involved in Ethanol-Induced Neuronal Oxidative Stress. **PLOS ONE**, v. 7, n. 5, p. e38075, 25 maio 2012.

WEISS, F. et al. Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. **The Journal of neuroscience : the official journal of the Society for Neuroscience**, v. 16, n. 10, p. 3474–3485, maio 1996.

WELDY, D. L. **Risks of alcoholic energy drinks for youth**. **Journal of the American Board of Family Medicine : JABFM** United States, 2010.

WELLS, B. E. et al. Correlates of concurrent energy drink and alcohol use among socially active adults. **The American journal of drug and alcohol abuse**, v. 39, n. 1, p. 8–15, jan. 2013.

WORLD HEALTH ORGANIZATION; MANAGEMENT OF SUBSTANCE ABUSE TEAM; WORLD HEALTH ORGANIZATION. **Global status report on alcohol and health 2018**. [s.l.: s.n.].

WU, D.; CEDERBAUM, A. I. Oxidative stress and alcoholic liver disease. **Seminars in liver disease**, v. 29, n. 2, p. 141–154, maio 2009.

ZALESKI, M. et al. Aspectos neurofarmacológicos do uso crônico e da Síndrome de Abstinência do Alcool. **Brazilian Journal of Psychiatry**, v. 26, p. 40–42, 2004.

5. ARTIGO

The effects of caffeine on alcohol oral self-administration behavior and prefrontal cortex DNA damage in rats

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ABSTRACT

Caffeine and alcohol are some of the most commonly used psychoactive substances in the world, and are often used concomitantly. However, little is known about the effect of caffeine on alcohol consumption. Here, our aim was to investigate the co-exposure of alcohol mixed with caffeine in self-administration. In addition, to verify the effect of caffeine on the damage index of DNA damage in the prefrontal cortex (CPF). Thirty-two male Wistar rats were randomly divided into the following groups: control, caffeine (0.25 mg / ml), alcohol (10% v / v) and alcohol mixed with caffeine (10% v / v + 0.25 mg / ml). During one week, the animals were submitted to the self-administration apparatus for habituation and training in the fixed ratio (FR1). Afterwards, they underwent self-administration for 21 days, being 1 hour per day. The forced swimming test (FST) was performed twice, the first one day before the training phase and the 24 hours after the last self-administration session. There was no significant difference in the volume of solution consumed to the control and caffeine groups. The volume of solution consumed in the alcohol group was lower than the control group and the caffeine group. Already the volume of solution consumed in the alcohol group mixed with caffeine was lower than the control, caffeine and alcohol groups. In the FST test, the caffeine group had a significant increase in immobility compared to the other groups. The alcohol mixed with caffeine group decreased immobility. The damage index (DI) for double-strand breaks was significantly higher in the alcohol group compared to the alcohol mixed

with caffeine and the caffeine groups. Our results showed a decrease in alcohol consumption when mixed with caffeine. There is great variability among similar studies on the effects of caffeine on alcohol consumption. Most used forced administration, which can produce significant behavioral changes. The self-administration model used here has a greater translational validity. In addition, Rezvani et al (2013) showed that the dose used may influence the effect of caffeine on consumption. In addition, we observed a lower DNA DI in animals that used caffeine when compared to the alcohol group. The main theory of this neuroprotective effect of caffeine is dose-dependent (Tiwari et al., 2014) and here the self-administration procedure proved valid to reproduce this effect.

INTRODUCTION

Alcohol consumption is a major public health problem, that draws attention both in terms of research and public policy. The World Health Organization (WHO) estimates that in 2016, more than 2.3 billion people aged 15 years and over routinely used alcoholic beverages, with a global average consumption of 6.4 liters of alcohol per capita year (World Health Organization et al. 2018). The implications of alcohol use itself do not only affect the health of users, they lead to risky behaviors such as traffic accidents, unprotected sex, trafficking and violence. Also, stopping alcohol use induces abstinence symptoms, that are the main reasons for relapse (McKinney and Coyle 2006; Penning et al. 2013).

The practice of drinking alcoholic beverages with caffeine is not new, but with the introduction of new sources of caffeine in the market this practice has become more common, mainly because of the emergence of energy drinks. These beverages contain high amounts of caffeine, ranging from 50 mg to 500 mg of caffeine per container (Reissig et al. 2009; Bryant Ludden and Wolfson 2010; Arria et al. 2011). Although energy drinks contain several components with psychoactive effects such as taurine and guarana. Studies have shown that caffeine is the active ingredient responsible for the behavioral and cognitive effects associated with these beverages (Attwood et al. 2012; Giles et al. 2012; Peacock et al. 2013b).

It is important to note that very little is known about the long-term consequences of caffeine and alcohol consumption. The literature evidences in humans that the consumption of alcohol mixed with highly caffeinated drinks is associated with a higher consumption of alcohol when compared to alcohol use alone (Ferreira et al. 2006; Marczinski and Fillmore 2006; Reissig et al. 2009; Velazquez et al. 2012; Marczinski et al. 2016). This effect is related to the fact that when consumed in combination, caffeine minimizes the aversive effects of alcohol on reaction time, short-term memory, wakefulness (Drake et al. 2003; Howland et al. 2011; Marczinski et al. 2012; Heinz et al. 2013), as well as increases subjective stimulation (Marczinski and Fillmore 2006; Marczinski 2011; Marczinski et al. 2012; Peacock et al. 2013a). Also, the use of caffeinated beverages with alcohol has been associated with greater use and a greater propensity for long-term alcohol dependence (Arria et al. 2011; Cheng et al. 2012). However, there is insufficient data to prove that caffeinated beverages directly increase alcohol consumption.

Animal studies have been employed to better understand the effects of caffeine on alcohol consumption. So far, the findings are mixed, with some studies demonstrating an increase in ethanol intake from the caffeine effect (Kunin et al. 2000; Rezvani et al. 2013), other studies do not demonstrate any caffeine effect (Hughes 2011; Fritz et al. 2014), and there

are studies showing a decrease in ethanol intake induced by caffeine (Rezvani et al. 2013). Co-administration of caffeine (5-40 mg / kg) had no effect on alcohol-induced decreases in learning in the task of discriminating avoidance of the high cross maze (Gulick and Gould 2009) or object recognition (Fritz et al. 2014). However, it has been observed that caffeine (5 mg / kg) reduces alcohol-induced retrograde memory loss in rats (Spinetta et al. 2008). Caffeine also did not alter the anxiolytic effects of alcohol in the elevated plus maze (8 mg / kg) (Fritz et al. 2014).

Calls ones attention that most rodent studies about co-exposure of caffeine and alcohol have used forced administration, systemic injections or intragastric gavages (Kunin et al. 2000; El Yacoubi et al. 2003; Ferreira et al. 2004; Spinetta et al. 2008; Gulick and Gould 2009; Hilbert et al. 2013; Rezvani et al. 2013). It is well-known that forced administrations can produce significant changes in behavior and neurobiology (Moolten and Kornetsky 1990; Jacobs et al. 2003; Mitchell et al. 2012) and that it does not elucidates the possible role of caffeine effects on the self-administration of alcohol. Therefore, self-administration models have greater translational validity in relation to human experience, either in respect to alcohol intoxication or to alcohol withdrawal.

In a recent study, we demonstrated that even low doses of alcohol may induce withdrawal behaviors in rats and neuronal DNA damage in several brain areas (Costa et al. 2015). Dna damage has been associated with ROS. Increased generation of ROS induced for alcohol are potentially harmful. Harper (Harper 2009) showed a neuronal loss in specific regions of the cortex, hypothalamus and cerebellum in alcoholics, and a significant increase of DNA damage was observed in these regions in rats with chronic alcohol use (Renis et al. 1996).

The objective of this study is to evaluate the consumption behavior of alcohol and caffeine, alone or in coadministration, and the consequences of this consumption in forced swimming behaviors after 24 hours of abstinence using a protocol of chronic oral self-administration. Additional objective was to measure the DNA damage resulting from the co-administration of alcohol and caffeine

METHODS

4.1 Animals

A sample of 32 male Wistar rats, young adults ($\pm$ 200 g, 55-65 PND), were obtained from the Animal House of Federal University of Health Sciences of Porto Alegre (UFCSPA). The animals were kept in polypropylene boxes (33 x 17 x 40 cm), 3 animals per box, under light / dark conditions of 12hs (7:00 AM to 7:00 PM lights on), temperature (22 ± 2 ° C) and humidity [55 %] controlled. All animals had

The animals were randomly allocated to their respective groups: Control, Caffeine, Alcohol and Alcohol mixed with Caffeine. During 7 days, the rats were trained to self-administer sucrose 2% using a FR1 schedule. On day 8, the self-administration for 21 days (FR1, 1 hour/day). The animals in the self-administration were in the same conditions of hours, apparatus and water restriction that went through the training. The Forced Swim Test (FST) was performed 1 day before the self-administration training and 24 hours after the last self-administration session. The animals performed the second FST under the same conditions as the first. Immediately after the last FST test the animals were sacrificed by guillotine in a sterile room and without direct light incidence and the prefrontal cortex was dissected and stored at -80 °C for later comet analysis.



For the alcohol group was used Ethanol (99.5 %v/v; Alphatec, Brazil) was diluted to 10 % (v/v)-the caffeine group prepared a solution of caffeine anhydrate base (0.25 mg / ml) and the alcohol groups with caffeine addition were prepared the 10% (v / v) ethanol solution and caffeine (0.25mg / ml). All solutions were

prepared with water and 2% sucrose (Synth, Brazil). Control groups received only the 2% sucrose solution.

4.3 Forced Swimming Test

The forced swimming test (FST) was performed two times. The first one, at the beginning of the experiments, Our protocol uses two consecutive days of behavioral observation (36, adapted from 37). On the first day the animals are introduced into an opaque glass aquarium (25 x 25 x 40 cm) filled with water at $25 \pm 1^{\circ}\text{C}$, and depth of 28 cm. for a 15 min training session, which aims to induce the state of behavioral despair or learned helplessness and to decrease the latency time for immobility (PORSOLT et al. 1977; Barros and Ferigolo 1998; Weiss et al. 1998). On the following day, the test session is performed, when the animal is exposed to the same condition above for a period of 5 minutes. After the self-administration phase of these experiments (28 days) with a 24 h period of drug abstinence all animal were retested in the FST for 5 min. All FST were recorded using a digital video camera for later detailed behavioral analysis.

4.4 Self-administration

The oral self-administration was performed in acrylic and metal apparatus (25x37x28 cm) containing on one of the walls 2 levers, of which only one is active ((Insight Ltda., Ribeirão Preto, SP, Brasil). Each self-administration apparatus is connected to an infusion pump containing a syringe.

The lever press on the active bar releases 0.2 ml of solution for 5 seconds on oral self-administration. The lever press in the bar during infusion would not produce a second infusion, only after the infusion term. The other bar is inactive during the session. Each session lasts 1 hour a day for 21 days. Training and self-management used a fixed ratio one by one (FR1)(Lopez and Becker 2014).

4.6 Comet Assay

Cells were suspended in a separate microtube with 300 μl of ice-cold PBS solution plus 20 mM EDTA and 10% DMSO. These tissues were macerated with a forceps. Concentration and cell viability were determined using trypan blue in Neubauer's chamber. The isolated cells were subsequently used to perform the comet assay in the neutral versions.

To prepare 20 μ l of the cell suspension were mixed with 90 μ l low-melting agarose solution (0.75%) and spread on pre-coated agarose (1.5%) microscope slides. The slides were incubated in ice-cold lysis solution (2.5 M NaCl, 10 mM Tris, 100 mM EDTA, 1% Triton X-100, and 10% DMSO, pH 10.0) at 4 ° C for 24 hours for removal of cell membranes. After this step, the slides were placed in a horizontal electrophoresis with the neutral buffer solution (100 mM Tris and 300 mM sodium acetate, pH 8.5) was incubated with a 60 min resting period of the slides and the electrophoresis was carried out for 1 hour in a current of 12 mA and a voltage of 14 V.

All steps described above were performed without direct incidence of light, in order to avoid further damage to the DNA molecule. After these steps, the slides were neutralized, after stained with silver nitrate for further observation under an optical microscope. One hundred cells from each treatment were selected for analysis of DNA migration, and the average of three slides of each sample was used to determine the damage index (DI). The DI is calculated score based on the classification of the different damage classes, which are visually determined according to the tail length in five classes: (1) Class 0: no damage, no tail; (2) Class 1: with a tail shorter than the head diameter of the head; (3) Class 2: with a tail length 1 - 2x the head diameter; (4) Class 3: with a tail longer than 2x the diameter of the head; and (5) Class 4: comets without heads. The DI ranges from 0 (without tail: 100 cells $\times$ 0) to 400 (with maximum migration: 100 cells $\times$ 4) (Costa et al. 2015; Freese et al. 2018).

4.7 Statistical analysis

Statistical analyzes were performed using statistical software Sigma-Stat 3.2 (Jandel, USA) and GraphPad Prism (La Jolla, CA, USA). The Kolmogorov-Smirnov test was used to verify the normality of all variables and for the verification of discrepant values the Grubbs test was used. Analyzes of variance for repeated measures (ANOVA) was used followed by the The Tukey's test as post-hoc when appropriate. Data are described as the mean $\pm$ standard error of the mean (SEM) and the differences were considered statistically significant when $p < 0.05$.

RESULTS

Our results demonstrate that alcohol, caffeine and alcohol plus caffeine are self-administered by rats for oral consumption leading to the use of quite important doses of the drugs during the one hour period of observation. Considering the volume of solutions and the weight of the animals it was possible to calculate the mean daily dose for each group. Considering alcohol intake, the animals that self-administered alcohol consumed (2.47 ± 0.052 g/kg/day) consumed significantly ($t= 3.45$; $p<0.005$) higher dose of alcohol in comparison to the alcohol and caffeine group (2.23 ± 0.044 g/kg/day). Considering caffeine intake, the animals that self-administered caffeine consumed (13.06 ± 0.14 mg/kg/day) consumed significantly ($t= 29.65$; $p<0.0001$) higher dose of alcohol in comparison to the alcohol mixed with caffeine group (7.08 ± 0.14 mg/kg/day)

In the Figure 1 we represent the mean of volume of drug consumption during the 21 days of drug self-administration. The control and caffeine group did not present significant differences in the amount consumed, in comparison to the control but presented higher consumption than the alcohol and caffeine alcohol groups. In comparison, the mean consumption of the alcohol group is significantly higher than the alcohol mixed with caffeine group

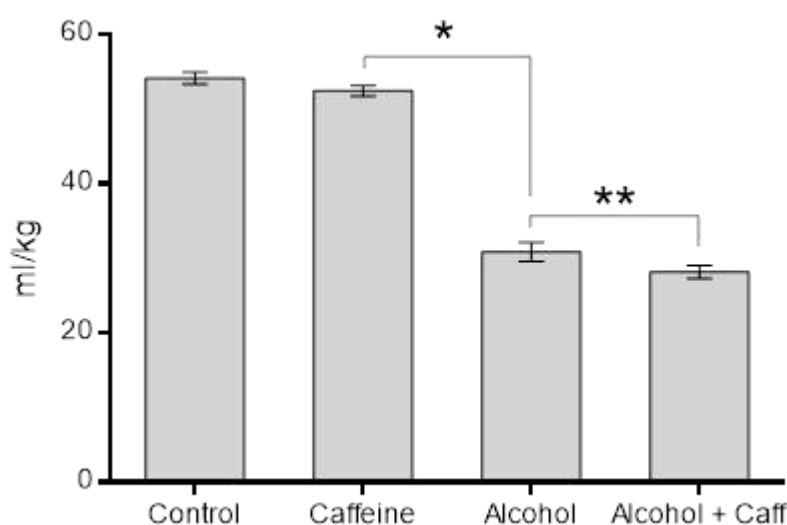


Figure 1: Total mean consumption ml/kg between groups during the 21 days. Data are presented as group means $\pm$ SEM. One-way ANOVA followed by Tukey's test were used

for statistical analysis. [$F(3, 491) = 579.5$ $P < 0.0001$]. * $p < 0.05$ compared to Control and Caffeine groups. * $p < 0.05$ compared to Alcohol. ** $p < 0.05$ compared to Alcohol+Caff group.

The daily mean consumption in ml/kg is shown in Figure 2. It may be observed that the consumption between the control and caffeine groups was uniform, during the 21 days. Only in one day the doses consumed had a slight difference, while the alcohol and alcohol mixed with caffeine has a greater variability, showing differences over several days.

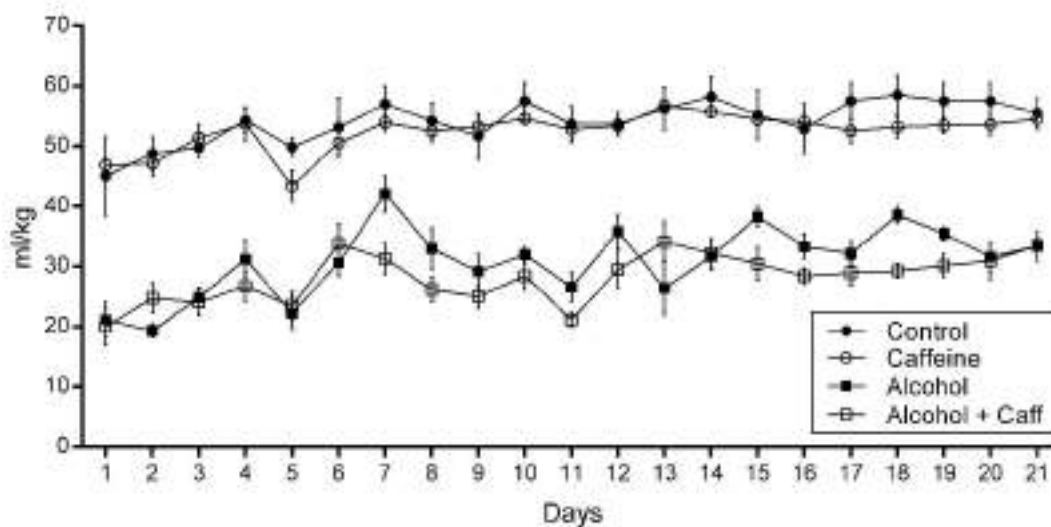


Figure 2: Daily mean consumption during the 21 days, Data are presented as group means $\pm$ SEM.

The number of lever press represented in Figure 3 shows a similar behavior between the control and caffeine animals, these two groups have a greater accumulated number of lever press in respect to the alcohol and alcohol mixed with caffeine groups from the 20mins on. The alcohol mixed with caffeine group begins to present a lower number of lever presses at the 30min of the session in respect to alcohol, and this difference is maintained until the end of our observation.

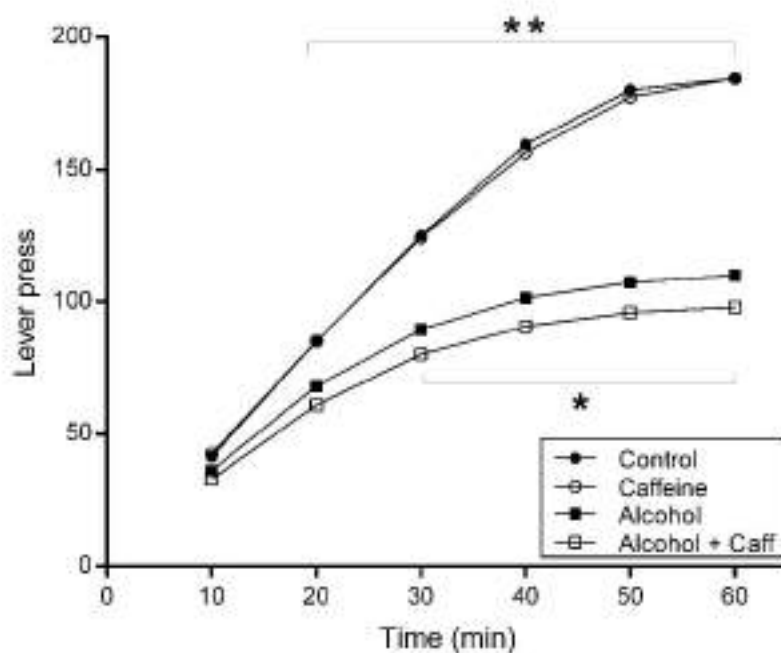


Figure 3: Mean of lever press accumulated per minute. Two-way ANOVA followed by Tukey's test were used for statistical analysis. ** $p < 0.001$ vs. Alcohol and Alcohol+Caff. * $p < 0.05$ compared to Alcohol+Caff group. [Lev. Pr = $F(3, 3456) = 1116$ $P < 0,0001$] [Time = $F(5, 3456) = 1652$ $P < 0,0001$] [Interaction $F(15, 3456) = 73,37$ $P < 0,0001$]

After the 21 days of oral self-administration, the animals were kept without drugs for 24 h before the forced swim. Figure 4 shows the immobility time of animals of the different groups after drug withdrawal in comparison to the first FST. The control and alcohol groups showed a slight but not significant increase in immobility, whereas the caffeine group had a considerable and significant increase in immobility represented by a difference from the other animals. Animals from the alcohol mixed with caffeine group decreased immobility, with significantly less immobility than the other groups. The biggest difference is between the caffeine and alcohol groups mixed with caffeine, while the control and alcohol groups are similar. There were no significant differences between the first and the last FST.

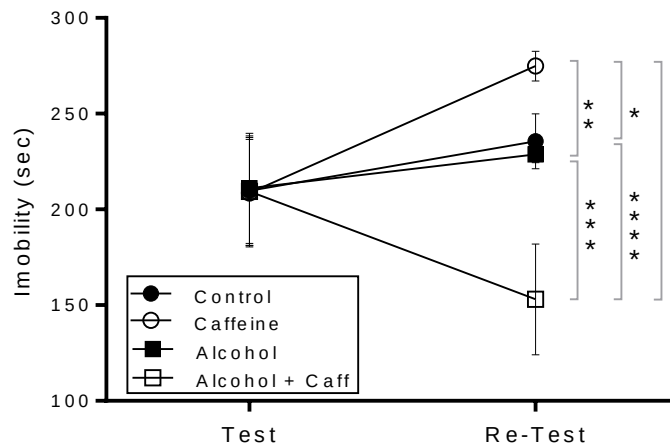


Figure 4: Mean of immobility of the initial and final FST. Data are presented as group means $\pm$ SEM. One-way ANOVA followed by Tukey's test were used for statistical analysis (Re-Test FST). ****p < 0.001 compared to Caffeine group. ****p < 0.001 compared to Alcohol+Caff group. ***p < 0.05 compared to Alcohol+Caff group. **p < 0.01 compared to Caffeine group. *p < 0.05 compared to Caffeine group. [F (3, 22) = 9,516 P = 0,0003].

The DNA damage index (DI) for double-strand breaks is presented in Figure 5. The DI was higher in the cortex of animals in the alcohol group, higher significantly compared to caffeine (P = 0.0251) and to the mixed alcohol and caffeine groups (P = 0.0181). The control group showed no difference between any other group. The lowest DI was from the alcohol group with caffeine, but it was only different from the alcohol group.

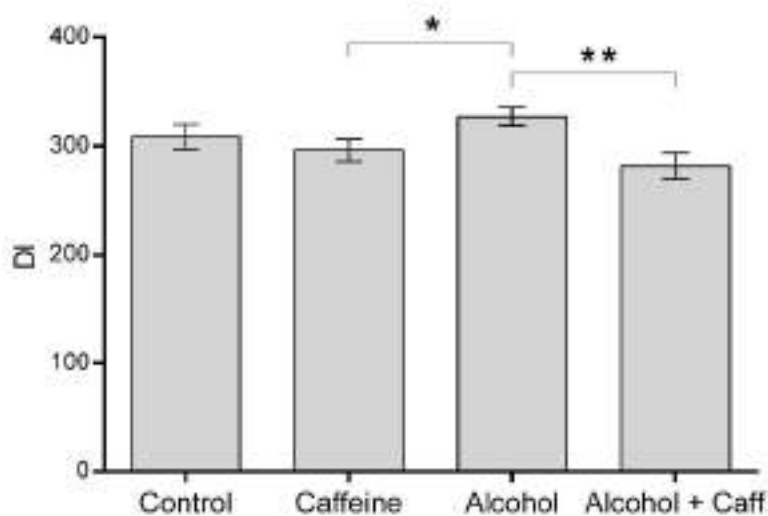


Figure 5: Mean of Damage Index (DI) of the cortex. Data are presented as group means $\pm$ SEM. One-way ANOVA followed by Tukey's test were used for statistical analysis. *p <0.05 compared to Caffeine group. **p <0.05 compared to Alcohol+Caff group.

DISCUSSION

This study was developed to verify if caffeine interacts with self-administration of alcohol and if it modifies the DNA damage induced by alcohol when alcohol and caffeine are co-administered. We verified that the animals regulate the amount of caffeine and of alcohol that is consumed by the oral route in a self-administration paradigm and the combined presentation of alcohol mixed with caffeine decreased the lever press for alcohol consumption and the amount of alcohol consumed. This decrease may be caused by some taste that caffeine may have in the solution. But it is also seen that in the caffeine group the animals presented higher behaviors and solution consumption than in the alcohol and caffeine combined solution. Newland (Newland and Brown 1992) showed that animals may consume up to 0.5 g / L of caffeine for oral solutions without alcohol.

This behavior of lower search for alcohol mixed with caffeine is reflected in the number of lever press and the cumulative lever pressure frequency by the animals. Because the lever presses started to decrease around 20min of the session when animals received oral alcohol or alcohol mixed with caffeine, one may suppose that the effects of alcohol symptoms is being expressed and as of 30min the self-administration in groups receiving alcohol mixed with caffeine was already lower than in the alcohol group. These results are comparable to those of caffeine (1g / L) in alcohol solutions (10% v / v) does not increase alcohol consumption in male rats (Carvalho et al. 2012). However, caffeine seems to have a dual effect on alcohol demand. At low doses of caffeine (5 mg / kg) shows an increase in alcohol consumption (Kunin et al. 2000; Rezvani et al. 2013), higher doses, between 10 and 20 mg / kg, may reduce alcohol intake (Kunin et al. 2000). Our results add information to the conflict of information in the literature, where 10mg/kg ip were seen not to have effect on alcohol intake (Rezvani et al. 2013) and oral 10 mg/kg of caffeine could reduce alcohol intake (Kunin et al. 2000). As our animals self-administering orally around to 13 and 7 mg/kg of caffeine used lower amounts of alcohol in a self-administration paradigm.

Caffeine abstinence presents a shorter time in ambulation (Nikodijević et al. 1993), but caffeine abstinence on immobility has not been fully elucidated. As to the

withdrawal behavioral changes induced by drugs it is seen that caffeine group animals showed more depressive-like behaviors during the abstinence. This result is in agreement with others studies showing that in caffeine abstinence less immobility is seen (Nehlig et al. 1992; Dews et al. 2002; Juliano and Griffiths 2004). This depressive-type behavior may also be accompanied by other withdrawal symptoms such as decreased energy, difficulty concentrating, decreased alertness, headache and tiredness (Griffiths and Woodson 1988; Juliano and Griffiths 2004). Caffeine withdrawal symptoms appear between 12-24h of abstinence and can last up to 6 days (Juliano and Griffiths 2004). Some studies suggest that caffeine withdrawal symptoms is a deregulation of the adenosinergic system (Nikodijević et al. 1993; Daly and Fredholm 1998), when this system is hyperstimulated daily causes an upregulation (Holtzman et al. 1991; Paul et al. 1993; Espinosa et al. 2013). In addition, other studies show an interaction between adenosine and dopamine receptors (Ferre et al. 1991, 1997; Okada et al. 1996), caffeine increases the release of dopamine, in abstinence the dopamine that was stimulated by adenosine is not present, causing type-like depressive symptoms (Okada et al. 1996; Solinas et al. 2002).

As in several other experiments in our lab (results not published) these moderate rats self-administering doses of alcohol for 21 days did not present changes in immobility. The abstinence from chronic alcohol consumption mixed with caffeine showing less time of immobility in the FST in this study needs to be elucidated. In mice, alcohol withdrawal-induced negative affect indicate the coincidence of increased anxiety-related behaviors on various behavioral tests with reduced immobility in the FST and this could also be the case in the present (Lee et al. 2017). The mechanistic hypothesis can be formulated by the effects that alcohol and caffeine have on the adenosinergic system, as adenosine receptor antagonist change the behaviors of chronic alcohol use on withdrawal (Gatch and Selvig 2002; El Yacoubi et al. 2003). Probably because changes to tolerance development (Batista et al. 2005). This relationship between alcohol and caffeine or other adenosine antagonists is complex and needs to be better investigated, especially in the withdrawal syndrome.

The ethanol-associated oxidative DNA damage in the central nervous system is well documented. Acute ethanol administration induce significant DNA damage in cerebellum and hippocampus and chronic administration of ethanol can induce

significant DNA damage in many more brain areas and the auto-repair of the DNA damage takes some days after ethanol withdrawal (Guo et al. 2007). It is already known that chronic alcohol exposure induce high DNA damage indexes the prefrontal cortex, hippocampus and striatum of rats in structures such as, and that this high DNA damage index is also seen after a short withdrawal from alcohol (Costa et al. 2015). Although the alcohol neuronal damage occurs during ethanol intoxication and not as a result of ethanol withdrawal (Manuscript et al. 2014). The DNA damage by alcohol response may be genetically influenced as mice genetically selected for divergent withdrawal severity present distinct neuroadaptive response during abstinence (Hashimoto et al. 2011). This might be the reason why the increase in DNA damage in the alcohol group in this study is less intense than the one seen in a previous group of animals in the same lab (Costa et al. 2015). Ethanol concurrently induces DNA damage, downregulates the DNA damage repair proteins, and increases apoptotic factors in hippocampal cells (Suman et al. 2016) and it is still necessary to verify which toxic mechanism lasts longer and is still active during withdrawal.

In this study, caffeine seems to have a protective effect towards alcohol, There was a lower DNA damage in the cortex of animals that used caffeine concomitantly with alcohol when compared to alcohol. To our knowledge, little is known about alcohol damage when given with caffeine and this seems to be the first study to address this question. Some studies point to caffeine having an antioxidant action interacting and accepting the hydroxyl radicals (Abreu et al. 2011), reducing lipid peroxidation, increasing glutathione concentration and increasing the activity of antioxidant enzymes glutathione reductase and superoxide dismutase (Abreu et al. 2011), inhibiting oxidative damage (Shi et al. 1991). Caffeine also expresses its neuroprotective effects through A2A receptor antagonism of glial cells, reducing microglial activation, inhibiting the release of cytokines, free radicals and glutamate (Sonsalla et al. 2012; Postuma et al. 2012; Bagga et al. 2016; Kolahehdouzan and Hamadeh 2017).

There is evidence that caffeine might decrease DNA damage in animals (Ferk et al. 2013) or in humans (Bakuradze et al. 2016). However, a recent study with comet assay of peripheral blood and hippocampal cells demonstrated no significant differences between caffeine-treated and control young adult mice in terms of DNA damage index (DI) and frequency while caffeine prevented DNA

damage in old mice (Damiani et al. 2017). The average dose self-administered by our animals is directly comparable to the doses that have an antioxidant effect. TIWARI (Tiwari et al. 2014) used two doses of caffeine (0.05 Mm and 0.1 Mm) in alveolar epithelial cells exposed to hyperoxia, at the lower dose (0.05 Mm) H₂O₂ levels decreased and (0.1 Mm) increased levels of H₂O₂.

In summary, this study provides references to complement new points of view in the literature. Currently in the literature, there are conflicts about the effect of caffeine on alcohol consumption, since there are different methodologies and different doses, in addition to factors such as the way caffeine is administered. Here, we seek the self-administration model with the aim of mimicking human use and choices. Our findings demonstrate that caffeine reduces intake and demand for alcohol when mixed with caffeine. In addition, animals that use caffeinated alcohol had a shorter immobility time. In DNA injury results, caffeine demonstrated neuroprotection, decreasing the damage index of DNA. This indicates that the role of caffeine in the doses used may be beneficial in alcohol consumption. Further study with different dosages should be made to better understand the co-exposure of caffeine and alcohol.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

References

- Abreu RV, Silva-Oliveira EM, Moraes MFD, et al (2011) Chronic coffee and caffeine ingestion effects on the cognitive function and antioxidant system of rat brains. *Pharmacol Biochem Behav* 99:659–664. doi: 10.1016/j.pbb.2011.06.010
- Arria AM, Caldeira KM, Kasperski SJ, et al (2011) Energy Drink Consumption and Increased Risk for Alcohol Dependence. *Alcohol Clin Exp Res* 35:365–375. doi: 10.1111/j.1530-0277.2010.01352.x
- Attwood AS, Rogers PJ, Ataya AF, et al (2012) Effects of caffeine on alcohol-related changes in behavioural control and perceived intoxication in light caffeine consumers. *Psychopharmacology (Berl)* 221:551–560. doi: 10.1007/s00213-011-2601-0
- Bagga P, Chugani AN, Patel AB (2016) Neuroprotective effects of caffeine in MPTP model of Parkinson's disease: A ¹³C NMR study. *Neurochem Int* 92:25–34. doi: 10.1016/j.neuint.2015.11.006
- Bakuradze T, Lang R, Hofmann T, et al (2016) Coffee consumption rapidly reduces background DNA strand breaks in healthy humans: Results of a short-term repeated uptake intervention study. *Mol Nutr Food Res* 60:682–686. doi: 10.1002/mnfr.201500668
- Barros HMT, Ferigolo M (1998) Ethopharmacology of imipramine in the forced-swimming test: Gender differences. *Neurosci Biobehav Rev* 23:279–286. doi: 10.1016/S0149-7634(98)00029-3
- Batista LC, Prediger RDS, Morato GS, Takahashi RN (2005) Blockade of adenosine and dopamine receptors inhibits the development of rapid tolerance to ethanol in mice. *Psychopharmacology (Berl)* 181:714–721. doi: 10.1007/s00213-005-0014-7
- Bryant Ludden A, Wolfson AR (2010) Understanding adolescent caffeine use: connecting use patterns with expectancies, reasons, and sleep. *Heal Educ Behav Off Publ Soc Public Heal Educ* 37:330–342. doi: 10.1177/1090198109341783
- Carvalho C, Silveira da Cruz J, Takahashi R (2012) Prolonged Exposure to Caffeinated Alcoholic Solutions Prevents the Alcohol Deprivation Effect in Rats.
- Cheng WJ, Cheng Y, Huang MC, Chen CJ (2012) Alcohol dependence, consumption of alcoholic energy drinks and associated work characteristics in the Taiwan working population. *Alcohol Alcohol* 47:372–379. doi: 10.1093/alcalc/ags034
- Costa PA, Poli JHZ, Sperotto NDM, et al (2015) Brain DNA damage and behavioral changes after repeated intermittent acute ethanol withdrawal by young rats. doi: 10.1007/s00213-015-4015-x
- Daly JW, Fredholm BB (1998) Caffeine--an atypical drug of dependence. *Drug Alcohol Depend* 51:199–206.
- Damiani AP, Garcez ML, Letieli de Abreu L, et al (2017) A reduction in DNA damage in neural tissue and peripheral blood of old mice treated with caffeine. *J Toxicol Environ Heal - Part A Curr Issues* 80:621–629. doi: 10.1080/15287394.2017.1286901
- Dews PB, Brien CPO, Bergman J (2002) Caffeine : behavioral effects of withdrawal and related issues. 40:1257–1261.
- Drake CL, Roehrs T, Turner L, et al (2003) Caffeine reversal of ethanol effects on the multiple sleep latency test, memory, and psychomotor performance. *Neuropsychopharmacology* 28:371–378. doi: 10.1038/sj.npp.1300026

- El Yacoubi M, Ledent C, Parmentier M, et al (2003) Caffeine reduces hypnotic effects of alcohol through adenosine A2A receptor blockade. *Neuropharmacology* 45:977–985.
- Espinosa J, Rocha A, Nunes F, et al (2013) Caffeine consumption prevents memory impairment, neuronal damage, and adenosine A2A receptors upregulation in the hippocampus of a rat model of sporadic dementia. *J Alzheimer's Dis* 34:509–518. doi: 10.3233/JAD-111982
- Ferk F, Huber WW, Grasl-kraupp B, Speer K (2013) Protective effects of coffee against induction of DNA damage and pre-neoplastic foci by aflatoxin B 1. 1–10. doi: 10.1002/mnfr.201300154
- Ferre S, Fredholm BB, Morelli M, et al (1997) Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. *Trends Neurosci* 20:482–487.
- Ferre S, von Euler G, Johansson B, et al (1991) Stimulation of high-affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. *Proc Natl Acad Sci U S A* 88:7238–7241.
- Ferreira SE, de Mello MT, Pompeia S, de Souza-Formigoni MLO (2006) Effects of energy drink ingestion on alcohol intoxication. *Alcohol Clin Exp Res* 30:598–605. doi: 10.1111/j.1530-0277.2006.00070.x
- Ferreira SE, Hartmann Quadros IM, Trindade AA, et al (2004) Can energy drinks reduce the depressor effect of ethanol? An experimental study in mice. *Physiol Behav* 82:841–847. doi: 10.1016/j.physbeh.2004.06.017
- Freese L, Almeida FB, Heidrich N, et al (2018) Environmental enrichment reduces cocaine neurotoxicity during cocaine-conditioned place preference in male rats. *Pharmacol Biochem Behav* 169:10–15. doi: 10.1016/j.pbb.2018.04.001
- Fritz BM, Companion M, Boehm SL (2014) “Wired,” Yet Intoxicated: Modeling Binge Caffeine and Alcohol Co-Consumption in the Mouse. *Alcohol Clin Exp Res* 38:2269–2278. doi: 10.1111/acer.12472
- Gatch MB, Selvig M (2002) Theophylline blocks ethanol withdrawal-induced hyperalgesia. *Alcohol Alcohol* 37:313–317.
- Giles GE, Mahoney CR, Brunye TT, et al (2012) Differential cognitive effects of energy drink ingredients: caffeine, taurine, and glucose. *Pharmacol Biochem Behav* 102:569–577. doi: 10.1016/j.pbb.2012.07.004
- Griffiths RR, Woodson PP (1988) Caffeine physical dependence: a review of human and laboratory animal studies. *Psychopharmacology (Berl)* 94:437–451.
- Gulick D, Gould TJ (2009) Effects of Ethanol and Caffeine on Behavior in C57BL/6 Mice in the Plus-Maze Discriminative Avoidance Task. *Behav Neurosci* 123:1271–1278. doi: 10.1037/a0017610
- Guo L, Wang LH, Sun B, et al (2007) Direct in vivo evidence of protective effects of grape seed procyanidin fractions and other antioxidants against ethanol-induced oxidative DNA damage in mouse brain cells. *J Agric Food Chem* 55:5881–5891. doi: 10.1021/jf070440a
- Harper C (2009) The neuropathology of alcohol-related brain damage. *Alcohol Alcohol* 44:136–140. doi: 10.1093/alcalc/agn102
- Hashimoto JG, Forquer MR, Tanchuck MA, et al (2011) Importance of genetic background for risk of relapse shown in altered prefrontal cortex gene expression during abstinence following chronic alcohol intoxication. *Neuroscience* 173:57–75. doi: 10.1016/j.neuroscience.2010.11.006

- Heinz AJ, de Wit H, Lilje TC, Kassel JD (2013) The combined effects of alcohol, caffeine, and expectancies on subjective experience, impulsivity, and risk-taking. *Exp Clin Psychopharmacol* 21:222–234. doi: 10.1037/a0032337
- Hilbert MLT, May CE, Grif WC (2013) Pharmacology , Biochemistry and Behavior Conditioned reinforcement and locomotor activating effects of caffeine and ethanol combinations in mice. 110:168–173. doi: 10.1016/j.pbb.2013.07.008
- Holtzman SG, Mante S, Minneman KP (1991) Role of adenosine receptors in caffeine tolerance. *J Pharmacol Exp Ther* 256:62–68.
- Howland J, Rohsenow DJ, Arnedt JT, et al (2011) The acute effects of caffeinated versus non-caffeinated alcoholic beverage on driving performance and attention/reaction time. *Addiction* 106:335–341. doi: 10.1111/j.1360-0443.2010.03219.x
- Hughes JE, Amyx H, Howard JL, et al (1994) Health effects of water restriction to motivate lever-pressing in rats. *Lab Anim Sci* 44:135–140.
- Hughes RN (2011) Adult anxiety-related behavior of rats following consumption during late adolescence of alcohol alone and in combination with caffeine. *Alcohol* 45:365–372. doi: 10.1016/j.alcohol.2010.10.006
- Jacobs EH, Smit AB, de Vries TJ, Schoffelmeer ANM (2003) Neuroadaptive effects of active versus passive drug administration in addiction research. *Trends Pharmacol Sci* 24:566–573. doi: 10.1016/j.tips.2003.09.006
- Juliano RLM, Griffiths RR (2004) A critical review of caffeine withdrawal : empirical validation of symptoms and signs , incidence , severity , and associated features. 1–29. doi: 10.1007/s00213-004-2000-x
- Kolahdouzan M, Hamadeh MJ (2017) The neuroprotective effects of caffeine in neurodegenerative diseases. *CNS Neurosci. Ther.* 23:272–290.
- Kunin D, Gaskin S, Rogan F, et al (2000) Caffeine promotes ethanol drinking in rats. Examination using a limited-access free choice paradigm. *Alcohol* 21:271–277.
- Lee KM, Coelho MA, Sern KR, et al (2017) Anxiolytic effects of buspirone and MTEP in the Porsolt Forced Swim Test. *Chronic Stress* (Thousand Oaks, Calif). doi: 10.1177/2470547017712985
- Lopez MF, Becker HC (2014) Operant ethanol self-administration in ethanol dependent mice. *Alcohol* 48:295–299. doi: 10.1016/j.alcohol.2014.02.002
- Manuscript A, Perception S, In O (2014) NIH Public Access. 218:759–785. doi: 10.1146/annurev-cellbio-092910-154240.Sensory
- Marczinski CA (2011) Alcohol mixed with energy drinks: consumption patterns and motivations for use in U.S. college students. *Int J Environ Res Public Health* 8:3232–3245. doi: 10.3390/ijerph8083232
- Marczinski CA, Fillmore MT (2006) Clubgoers and their trendy cocktails: implications of mixing caffeine into alcohol on information processing and subjective reports of intoxication. *Exp Clin Psychopharmacol* 14:450–458. doi: 10.1037/1064-1297.14.4.450
- Marczinski CA, Fillmore MT, Henges AL, et al (2012) Effects of energy drinks mixed with alcohol on information processing, motor coordination and subjective reports of intoxication. *Exp Clin Psychopharmacol* 20:129–138. doi: 10.1037/a0026136
- Marczinski CA, Fillmore MT, Stamates AL, Maloney SF (2016) Desire to Drink Alcohol is Enhanced with High Caffeine Energy Drink Mixers. *Alcohol Clin Exp Res* 40:1982–1990. doi: 10.1111/acer.13152

- McKinney A, Coyle K (2006) Alcohol hangover effects on measures of affect the morning after a normal night's drinking. *Alcohol Alcohol* 41:54–60. doi: 10.1093/alcalc/agh226
- Mitchell JM, Margolis EB, Coker AR, Fields HL (2012) Alcohol self-administration, anxiety, and cortisol levels predict changes in delta opioid receptor function in the ventral tegmental area. *Behav Neurosci* 126:515–522. doi: 10.1037/a0029027
- Moolten M, Kornetsky C (1990) Oral self-administration of ethanol and not experimenter-administered ethanol facilitates rewarding electrical brain stimulation. *Alcohol* 7:221–225. doi: 10.1016/0741-8329(90)90008-Z
- Nehlig A, Daval JL, Debry G (1992) Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Res Brain Res Rev* 17:139–170.
- Newland MC, Brown K (1992) Oral caffeine consumption by rats: the role of flavor history, concentration, concurrent food, and an adenosine agonist. *Pharmacol Biochem Behav* 42:651–659.
- Nikodijević O, Jacobson KA, Daly JW (1993) Locomotor activity in mice during chronic treatment with caffeine and withdrawal. *Pharmacol Biochem Behav* 44:199–216. doi: 10.1016/0091-3057(93)90299-9
- Okada M, Mizuno K, Kaneko S (1996) Adenosine A1 and A2 receptors modulate extracellular dopamine levels in rat striatum. *Neurosci Lett* 212:53–56.
- Paul S, Kurunwune B, Biaggioni I (1993) Caffeine withdrawal: apparent heterologous sensitization to adenosine and prostacyclin actions in human platelets. *J Pharmacol Exp Ther* 267:838–843.
- Peacock A, Bruno R, Martin FH (2013a) Patterns of use and motivations for consuming alcohol mixed with energy drinks. *Psychol Addict Behav* 27:202–6. doi: 10.1037/a0029985
- Peacock A, Martin FH, Carr A (2013b) Energy drink ingredients. Contribution of caffeine and taurine to performance outcomes. *Appetite* 64:1–4. doi: 10.1016/j.appet.2012.12.021
- Penning R, McKinney A, Bus LD, et al (2013) Measurement of alcohol hangover severity: development of the Alcohol Hangover Severity Scale (AHSS). *Psychopharmacology (Berl)* 225:803–810. doi: 10.1007/s00213-012-2866-y
- PORSOLT RD, LE PICHON M, JALFRE M (1977) Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266:730–732. doi: 10.1038/266730a0
- Postuma RB, Lang AE, Munhoz RP, et al (2012) Caffeine for treatment of Parkinson disease: a randomized controlled trial. *Neurology* 79:651–658. doi: 10.1212/WNL.0b013e318263570d
- Reissig CJ, Strain EC, Griffiths RR (2009) Caffeinated energy drinks-A growing problem. *Drug Alcohol Depend* 99:1–10. doi: 10.1016/j.drugalcdep.2008.08.001
- Renis M, Calabrese V, Russo A, et al (1996) Nuclear DNA strand breaks during ethanol-induced oxidative stress in rat brain. *FEBS Lett* 390:153–156.
- Rezvani AH, Sexton HG, Johnson J, et al (2013) Effects of caffeine on alcohol consumption and nicotine self-administration in rats. *Alcohol Clin Exp Res* 37:1609–1617. doi: 10.1111/acer.12127
- Shi X, Dalal NS, Jain AC (1991) Antioxidant behaviour of caffeine: efficient scavenging of hydroxyl radicals. *Food Chem Toxicol* 29:1–6.

- Solinas M, Ferre S, You Z-B, et al (2002) Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. *J Neurosci* 22:6321–6324. doi: 20026640
- Sonsalla PK, Wong L-Y, Harris SL, et al (2012) Delayed caffeine treatment prevents nigral dopamine neuron loss in a progressive rat model of Parkinson's disease. *Exp Neurol* 234:482–487. doi: 10.1016/j.expneurol.2012.01.022
- Spinetta MJ, Woodlee MT, Feinberg LM, et al (2008) Alcohol-induced retrograde memory impairment in rats: prevention by caffeine. *Psychopharmacology (Berl)* 201:361–371. doi: 10.1007/s00213-008-1294-5
- Suman S, Kumar S, N'Gouemo P, Datta K (2016) Increased DNA double-strand break was associated with downregulation of repair and upregulation of apoptotic factors in rat hippocampus after alcohol exposure. *Alcohol* 54:45–50. doi: 10.1016/j.alcohol.2016.06.003
- Tiwari KK, Chu C, Couroucli X, et al (2014) Differential concentration-specific effects of caffeine on cell viability, oxidative stress, and cell cycle in pulmonary oxygen toxicity in vitro. *Biochem Biophys Res Commun* 450:1345–1350. doi: 10.1016/j.bbrc.2014.06.132
- Toth L a, Gardiner TW (2000) Food and water restriction protocols: physiological and behavioral considerations. *Contemp Top Lab Anim Sci* 39:9–17.
- Velazquez CE, Poulos NS, Latimer LA, Pasch KE (2012) Associations between energy drink consumption and alcohol use behaviors among college students. *Drug Alcohol Depend* 123:167–172. doi: 10.1016/j.drugalcdep.2011.11.006
- Weiss JM, Cierpial M a, West CH (1998) Selective breeding of rats for high and low motor activity in a swim test: toward a new animal model of depression. *Pharmacol Biochem Behav* 61:49–66. doi: 10.1016/S0091-3057(98)00075-6
- World Health Organization, Management of Substance Abuse Team, World Health Organization (2018) Global status report on alcohol and health 2018.

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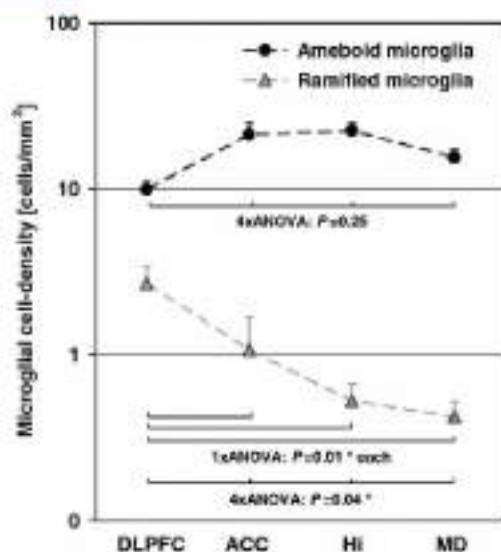
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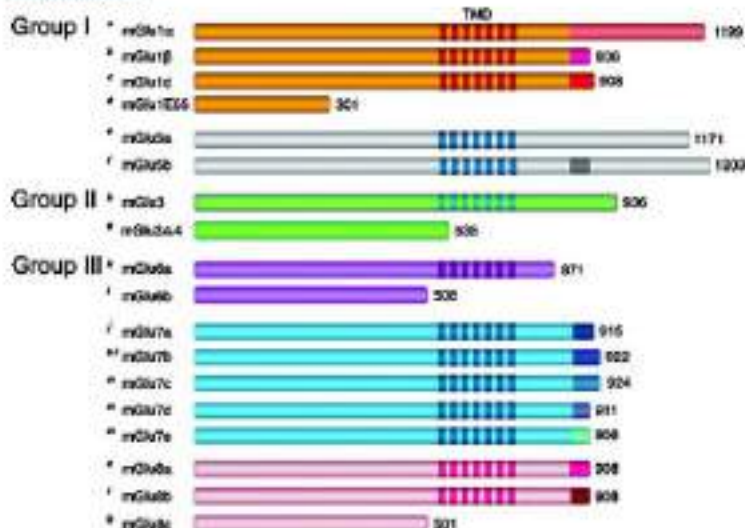
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PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA E ENSINO

1) PROTOCOLO Nº: 214/17

2) DATA DO PARECER: 11/10/2017

Parecer 525/17

3) TÍTULO DO PROJETO:

Efeitos da cafeína na toxicidade e na autoadministração de álcool em ratos com comportamentos tipo depressivo.

4) PESQUISADOR RESPONSÁVEL:

Helena Maria Tannhauser Barros

5) RESUMO DO PROJETO:

O projeto visa analisar efeitos da cafeína na toxicidade e na autoadministração de álcool em ratos com comportamento tipo depressivo. O teste do nado forçado será utilizado para separação dos animais quanto ao comportamento tipo depressivo ou não. Será realizada a análise de transcrição das subunidades GABA α em regiões do hipocampo e lesões no DNA através de teste cometa.

6) OBJETIVOS DO PROJETO:

Objetivo geral:

Avaliar o comportamento de consumo de álcool e os efeitos comportamentais induzidos por álcool em ratos com baixo e alto comportamento tipo depressivo (imobilidade no FST), expostos ou não expostos a cafeína.

Objetivos específicos:

- 1- Avaliar se a exposição a cafeína modifica comportamentos induzidos pelo álcool e comportamentos tipo depressivos em ratos com baixa imobilidade no teste do nado forçado.
- 3- Avaliar se a exposição à cafeína modifica comportamentos induzidos pelo álcool e comportamentos tipos depressivos em ratos com alta imobilidade no teste do nado forçado.
- 4- Verificar se há diferença na abstinência ao álcool entre os grupos de animais com alta e baixa imobilidade no teste do nado forçado.
- 5 – Verificar se há diferença na abstinência ao álcool entre os grupos de animais com alta e baixa imobilidade expostos ao álcool associado ou não com cafeína.
- 6- Avaliar o efeito do álcool e cafeína sobre o sistema GABAérgico, através da quantificação da expressão de mRNA de subunidades do receptor GABA A, em diferentes regiões cerebrais relacionadas com o sistema límbico.

7 – Comparar os danos no DNA entre os grupos em diferentes regiões cerebrais em animais expostos ao álcool associado ou não com cafeína.

7) FINALIDADE DO PROJETO: ☐ Ensino ☒ Pesquisa

8) ITENS METODOLÓGICOS E ÉTICOS DO PROJETO:

Título ☒ Adequado ☐ Comentários

Introdução ☒ Adequada ☐ Comentários

Objetivos ☒ Adequados ☐ Comentários

Relevância e Justificativa ☒ Adequados ☐ Comentários

Materiais e Métodos ☒ Adequados ☐ Comentários

Cronograma para execução da pesquisa ☒ Adequado ☐ Comentários

Orçamento e fonte financiadora ☒ Adequados ☐ Comentários

Referências Bibliográficas ☒ Adequadas ☐ Comentários

9) O PROJETO ESTÁ ADEQUADO À LEGISLAÇÃO VIGENTE:

☒ Sim ☐ Não

10) INFORMAÇÕES RELATIVAS AOS ANIMAIS:

Grau de dor/estresse: B ☒ C ☐ D ☐ E ☐

Justifique:

Espécie: *Ratus norvegicus* - Wistar

Número Amostral: 128



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Redução Amostral:

☐ Sim

☒ Não

Justifique:

Substituição de Metodologia:

☐ Sim

☒ Não

Se achar necessário, justifique e sugira uma nova metodologia:

Aprimoramento da Metodologia:

☐ Sim

☒ Não

Se achar necessário, justifique e sugira aprimoramentos da metodologia:

Acomodação e manutenção dos animais:

☒ Adequada

☐ Inadequada

Se achar inadequada cite abaixo as melhorias necessárias:

Manipulação dos animais:

☒ Adequada

☐ Inadequada

Se achar inadequada cite abaixo as melhorias necessárias:

Analgesia dos animais (se aplicável):

☐ Adequada

☐ Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com analgésico substituído:

Anestesia dos animais (se aplicável):

☐ Adequada

☐ Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com anestésico substituído:

Eutanásia dos animais (se aplicável):

☒ Adequada

☐ Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com metodologia substituída:

Comentários abaixo.

Local de Realização (Biotério/Laboratório): Laboratório de Neuropsicofarmacologia e Biotério da UFCSPA.

Outra instituição. Qual?

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11) CRONOGRAMA DE UTILIZAÇÃO DE ANIMAIS

Data	Espécie	Sexo	Quantidade
Dezembro de 2017	Ratos Wistar	Masculino e feminino	64 de cada sexo = 128 animais

12) RECOMENDAÇÃO:

☒ Aprovado

☐ Com Pendência

☐ Não aprovado

01/03/2017

Data de início

30/02/2019

Data de Término

Comentários gerais sobre o projeto:

As questões levantadas foram respondidas adequadamente.

Sendo assim, o presente projeto está aprovado pelo CEUA-UFCSPA.