UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA

Gerson Fernandes de Brum

EFEITO DO TRATAMENTO COM ÁCIDO GÁLICO SOBRE PARÂMETROS OXIDATIVOS, MOLECULARES E COMPORTAMENTAIS INDUZIDOS POR CETAMINA EM RATOS

Santa Maria, RS
2019

Gerson Fernandes de Brum

EFEITO DO TRATAMENTO COM ÁCIDO GÁLICO SOBRE PARÂMETROS OXIDATIVOS, MOLECULARES E COMPORTAMENTAIS INDUZIDOS POR CETAMINA EM RATOS

Dissertação apresentada ao Curso de Pós-Graduação em Farmacologia, Área de Concentração em Neuropsicofarmacologia e Imunofarmacologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Farmacologia.**

Orientadora: Profª. Dra. Marilise Escobar Burger

Santa Maria, RS

2019

de Brum, Gerson Fernandes
EFEITO DO TRATAMENTO COM ÁCIDO GÁLICO SOBRE PARÂMETROS OXIDATIVOS, MOLECULARES E COMPORTAMENTAIS INDUZIDOS POR CETAMINA EM RATOS / Gerson Fernandes de Brum.- 2019.
64 p.; 30 cm

Orientadora: Marilise Escobar Burger
Dissertação (mestrado) - Universidade Federal de Santa Maria, Centro de Ciências da Saúde, Programa de Pós Graduação em Farmacologia, RS, 2019

1. Neurotoxicidade 2. Memória de trabalho 3. Hipocampo 4. Droga aditiva 5. Antioxidante I. Escobar Burger, Marilise II. Título.

Sistema de geração automática de ficha catalográfica da UPSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

Gerson Fernandes de Brum

EFEITO DO TRATAMENTO COM ÁCIDO GÁLICO SOBRE PARÂMETROS OXIDATIVOS, MOLECULARES E COMPORTAMENTAIS INDUZIDOS POR CETAMINA EM RATOS

Dissertação apresentada ao Programa de Pós-Graduação em Farmacologia, Área de concentração em Neuropsicofarmacologia e Imunofarmacologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Farmacologia.**

Aprovado em 20 de dezembro de 2019:

Marilise Escobar Bürger, Dra. (UFSM)
(Presidente/Orientadora)

Virgínia Cielo Rech, Dra. (UFN)

Heeson Jesser Segat, Dr. (UFSM)

Santa Maria, RS
2019

AGRADECIMENTOS

Agradeço a todos que contribuíram para a conclusão deste estudo e, de uma forma especial, agradeço:

- à Deus e ao meu anjo da guarda que me protegem e acompanham todos os dias.

- à minha família por todo apoio e cuidado.

- ao Matheus Silveira Jardim meu confidente, amigo e companheiro de todas as horas.

- à minha orientadora, professora Marilise Escobar Burger, a quem manifesto minha gratidão por toda experiência pessoal e profissional adquirida no mestrado.

- à minha amiga-irmã Kátia Nascimento pelo incentivo em realizar o mestrado.

- as minhas amigas cujo a Biomedicina uniu para toda vida (Camila dos Santos Ritter, Brenda Moreira dos Santos e Cristiane Antunes Teixeira).

- aos meus colegas e amigos do laboratório Farmatox por toda ajuda durante o desenvolvimento dos experimentos e da escrita (Vinícia Garzela Metz, Laura Hautrive Milanesi, Higor Zuquetto Rosa, Domenika Rubert Rossato e Jéssica Leandra da Rosa).

- e finalmente, a Universidade Federal de Santa Maria por proporcionar o conhecimento científico por intermédio de uma pós-graduação.

Muito obrigado!

‘‘Não há caminho errado.
O aprendizado e a experiência estão em todos os caminhos’’.

(Zíbia Gasparetto)

RESUMO

EFEITOS DO TRATAMENTO COM ÁCIDO GÁLICO SOBRE PARÂMETROS OXIDATIVOS, MOLECULARES E COMPORTAMENTAIS INDUZIDOS POR CETAMINA EM RATOS

AUTOR: Gerson Fernandes de Brum
ORIENTADORA: Profª. Dra. Marilise Escobar Burger

A cetamina é um anestésico dissociativo que bloqueia receptores glutamatérgicos NMDA, de uso clínico restrito devido ao alto potencial de abuso. Estudos têm mostrado o desenvolvimento de neurotoxicidade e prejuízos de memória frente a exposição aguda e crônica à cetamina. O ácido gálico (AG) é um antioxidante natural, cuja atividade têm mostrado benefícios sobre a memória. O presente estudo foi desenvolvido para avaliar possíveis ações benéficas do AG sobre as consequências nocivas decorrentes da administração aguda e subcrônica de cetamina em ratos machos adolescentes. Quarenta e cinco ratos Wistar foram distribuídos em dois protocolos experimentais (UFSM/8629131218) no protocolo 1 (exposição aguda tipo *binge* - Binge-KET), animais receberam cinco doses de cetamina (KET) (10 mg / kg, ip) a cada três horas nas primeiras 12h do dia, recebendo três doses de AG (13,5 mg / kg, po) ou veículo nas 12 horas seguintes; no protocolo 2 (exposição subcrônica a KET - SbChro-KET), os animais receberam uma dose díaria de KET (10 mg/kg, ip) durante 10 dias, sendo posteriormente tratados com uma dose diária de AG (13,5 mg / kg, po) ou veículo por três dias consecutivos. No protocolo I, a KET prejudicou a memória de trabalho dos animais ao reduzir o percentual de alternância no teste do labirinto em Y. Análises bioquímicas mostraram que estes animais apresentaram níveis aumentados de creatinina no plasma, de lipoperoxidação (LP) no rim e hipocampo, além de aumentada atividade da catalase (CAT) no fígado e hipocampo, a qual foi reduzida no rim. A exposição aguda à KET também modificou parâmetros moleculares, elevando a imunoreatividade hipocampal de pro-BDNF e TrkB. O tratamento com AG não reverteu a memória prejudicada pela KET aguda, porém reverteu totalmente os niveis de creatinina (plasma), da atividade da CAT (fígado e hipocampo) e da LP (rim), além de reverter o imunoconteúdo de pré-BDNF e TrKB. No protocolo II, os animais subcronicamente expostos a KET mostraram prejuízos de memória, aumentados níveis de AST e ALT no plasma, de LP no fígado, rim e hipocampo, além de aumentada atividade da CAT no fígado e rim. À nível molecular, tal exposição à KET elevou a imunorreatividade do pró-BDNF e reduziu os níveis de BDNF e TrkB no hipocampo. O AG recuperou a memória de trabalho, antes prejudicada pela droga no protocolo agudo, recuperou totalmente os níveis de AST e ALT no plasma, e também da LP no rim, fígado e hipocampo além da atividade da CAT no fígado e rim. Todas a alterações moleculares hipocampais (pró-BDNF, BDNF e TrkB) induzidas pela exposição subcrônica à KET foram revertidas pelo AG. Interessantemente, os níveis de BDNF mostraram correlação negativa com níveis de LP no hipocampo e positiva com o percentual de alternância no labirinto-Y, enquanto este último mostrou correlação negativa com os níveis hipocampais de LP. Diante destes resultados, é possível sugerir que, após estudos clínicos, o AG poderá ser considerado um agente antioxidante natural de utilidade promissora para reversão de danos induzidos pelo uso abusivo tanto agudo como subcrônico de KET.

Palavras-chave: Neurotoxicidade. Memória de trabalho. Hipocampo. Droga aditiva. Antioxidante**.**

ABSTRACT

EFFECTS OF GALIC ACID TREATMENT ON OXIDATIVE, MOLECULAR AND BEHAVIORAL PARAMETERS INDUCED TO KETAMINE IN RATS

AUTHOR: Gerson Fernandes de Brum
ADVISOR: Profª. Dra. Marilise Escobar Burger

Ketamine is a dissociative anesthetic drug, which blocks NMDA glutamatergic receptors for restricted clinical use due to its high addictive potential. Studies have shown neurotoxicity and memory impairments development after acute- and chronic-KET exposure. Gallic acid (AG) is a natural antioxidant, whose activity has shown benefits over memory. The current study was designed to evaluate possible beneficial actions of GA on the harmful consequences of acute and sub-chronic ketamine administration in male adolescent rats. Forty-five Wistar rats were assigned to two experimental protocols (UFSM/8629131218): In the protocol 1 (acute/binge exposure; Binge-KET group), animals received five doses of KET (ketamine) (10 mg/kg, ip) every three hours for the first 12 hours of the day, receiving three doses of GA (13.5 mg/ kg, po) or vehicle in the following 12 hours; In protocol 2 (sub-chronic exposure to KET; SbChro-KET group), animals received a daily dose of KET (10 mg/kg, ip) for 10 days and they were sequentially treated with one dose of GA (13.5 mg/kg, po) or vehicle daily for three consecutive days. In protocol I, KET impaired the working memory of the animals by reducing the percentage of alternation in the Y labyrinth test. Biochemical analyzes showed that these animals showed increased levels of plasma creatinine, lipoperoxidation (LP) in the kidney and hippocampus, in addition to increased catalase (CAT) activity in the liver and hippocampus, which was reduced in the kidney. Acute exposure to KET also changed molecular parameters, increasing hippocampal immunoreactivity of pro BDNF and TrkB. Treatment with AG did not reverse the impaired memory by acute KET, but it completely reversed the levels of creatinine (plasma), CAT activity (liver and hippocampus) and LP (kidney), in addition to reversing the immunocontent of pre BDNF and TrKB. In protocol II, animals subchronically exposed to KET showed impaired memory, increased levels of AST and ALT in plasma, LP in the liver, kidney and hippocampus, in addition to increased CAT activity in the liver and kidney. At the molecular level, such exposure to KET increased the pro-BDNF immunoreactivity and reduced the levels of BDNF and TrkB in the hippocampus. The AG recovered working memory, previously impaired by the drug in the acute protocol, fully recovered the levels of AST and ALT in plasma, and also of LP in the kidney, liver and hippocampus in addition to CAT activity in the liver and kidney. All hippocampal molecular changes (pro BDNF, BDNF and TrkB) induced by subchronic KET exposure were reversed by AG. Interestingly, BDNF levels showed a negative correlation with LP levels in the hippocampus and positive with the percentage of alternation in the Y labyrinth, while the latter showed a negative correlation with the hippocampal levels of LP**.** Given these outcomes, it is possible to suggest that, following clinical studies, GA may be considered a natural antioxidant agent of promising usefulness for reversing damages induced by both acute and subchronic-KET abuse.

Keywords: Neurotoxicity. Working memory. Hippocampus. Additive drug. Antioxidant.

LISTA DE FIGURAS

Figura 1. Estrutura química dos enantiômeros de cetamina.....15

Figura 2. Estrutura química do ácido gálico.....19

Figuras do manuscrito:

Figure 1. Experimental design……...47

Figure 2.Y-maze task……48

Figure 3. pro-BDNF, BDNF and TrkB immunoreactivity…….49

Figure 4. Correlations…….....50

LISTA DE TABELAS

Table 1. Biochemical parameters……....51

Table 2. Oxidative stress parameters……...52

LISTA DE ABREVIATURAS E SIGLAS

AG	Ácido Gálico
ALT	Alanina aminotransferase
AST	Aspartato aminotransferase
BDNF	Fator Neurotrófico Derivado do Cérebro (do inglês: *Brain-derived neurotrophic factor*)
Binge-KET	Exposição aguda de cetamina
EO	Estresse oxidativo
ERs	Espécies Reativas
FDA	Agência de Controle de Alimentos e Medicamentos (do inglês: *Food and Drug Administration*)
KET	Cetamina (do inglês: Ketamine)
MAO	Monoamina oxidase
NMDA	N-metil D-Aspartato
Pro-BDNF	Precursor do BDNF
SbChro-KET	Cetamina subcrônica
SNC	Sistema Nervoso Central
TrkB	Receptor de tropomiosina quinase B (do inglês: *Tropomyosin receptor kinase B*)

SUMÁRIO

1. INTRODUÇÃO 12
2. OBJETIVOS 13
2.1 Objetivos gerais 13
2.2 Objetivos específicos 13
3. REVISÃO BIBLIOGRÁFICA 14
3.1 Contexto histórico da cetamina 14
3.2 Cetamina: farmacocinética e farmacodinâmica 15
3.3 Cetamina e sistema cognitivo 16
3.4 Estresse oxidativo e cetamina 17
3.5 Ácido gálico e suas propriedades terapêuticas 18
4. JUSTIFICATIVA 21
5. DESENVOLVIMENTO 22
5.1 Manuscrito científico 22
6. CONCLUSÃO 53
REFERÊNCIAS 54
ANEXO I – CERTIFICADO DE APROVAÇÃO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA UNIVERSIDADE FEDERAL DE SANTA MARIA 64

APRESENTAÇÃO

Esta dissertação está estruturada em seções dispostas em: Introdução, Objetivo Revisão bibliográfica, Justificativa, Desenvolvimento (Manuscrito Científico), Conclusões e Referências.

Os itens Materiais e Métodos, Resultados, Discussão dos resultados e suas Referências encontram-se inseridos na seção Desenvolvimento, subseção Manuscrito Científico**,** representando a íntegra deste estudo.

As Referências (pag. 54) referem-se somente às citações que aparecem na seção Introdução e Revisão bibliográfica desta dissertação.

1. INTRODUÇÃO

A cetamina (KET) é um fármaco anestésico dissociativo cujo mecanismo de ação ocorre através do bloqueio não competitivo do receptor glutamatérgico N-metil-D-aspartato (NMDA) (GARCIA, 2007). Geralmente, a KET é disponível na forma racêmica de dois enantiômeros, o R (-) e o S (+), sendo amplamente utilizada na clínica humana e veterinária em procedimentos cirúrgicos (ANNETA, et al., 2005). Além da anestesia geral, o enantiômero S (+) da KET (esketamina) foi recentemente liberado o pelo *Food and Drug Administration* (FDA- E.U.A.) para tratamento agudo de pacientes com alto grau de depressão, com potencial suicida, e que não respondem a outros medicamentos. No entanto, neste contexto de utilização clínica, a KET é classificada como droga de potencial abusivo devido ao desenvolvimento de efeitos hedônicos (BASCUÑANA et al., 2003; FDA, 2019), o que levou ao comércio restrito ao ambiente hospitalar, pois seu uso ilícito favorece o desenvolvimento de dependência (MORGAN & CURRAN, 2012). Contudo, infelizmente o uso abusivo da KET apresenta um crescimento considerável, o que compromete os já escassos recursos da saúde pública, colocando em risco a vida dos usuários (SILVA, 2010; WDR, 2018, UNODC, 2014).

Diferentes estudos têm demonstrado que a KET provoca inúmeros danos aos órgãos periféricos (fígado e rim) e ao sistema nervoso central (SNC). (DU et al. 2017; ZUGNO et al., 2013; YADAV et al., 2017; CARTÁGENES et al., 2019; BAKER et al., 2016). Evidências apontam que a exposição aguda a essa substância pode causar danos sobre a memória e a locomoção (HUANG et al. 2012; PAULEet al. 2011, SORIANO et al. 2010; ZOU et al. 2009; CRAWFORD et al, 2019), enquanto o uso crônico pode induzir além desses mesmos prejuízos comportamentais, o desenvolvimento de estresse oxidativo (EO) e neuroinflamação (LI, et al., 2017;YADAV et al., 2017).

O ácido gálico (AG) (ácido 3,4,5-tri-hidroxibenzóico), é um antioxidante natural encontrado especialmente no chá verde (*Camellia sinensis*) e em frutas como o caju (*Anacardium occidentale)* e a gabiroba (*Campomanesia xanthocarpa*) (LU et al., 2006; ROCHA, et al., 2011), desempenhando um papel promissor frente a substâncias neurotóxicas e pró-oxidantes como a KET (YADAV et al., 2017). Além disso, o AG também apresenta propriedade antidepressiva, antinflamatória, anti-tumoral, além de prevenir déficits de memória (CHHILLAR; DHINGRA, 2012; SARKAKI et al. 2015, SAFAEI et al. 2018; DAGLIA et al., 2014; LIMA et al., 2016, FARBOOD et al., 2013;). Sendo assim, o AG torna-se uma alternativa a ser estudada como tratamento após exposições abusivas ou repetidas de KET.

2. OBJETIVOS

2.1 OBJETIVOS GERAIS

Avaliar os efeitos do tratamento com AG sobre parâmetros comportamentais, oxidativos e moleculares induzidos pela administração aguda tipo *binge* e subcrônica de KET em ratos adolescentes.

2.2 OBJETIVOS ESPECÍFICOS

- Avaliar a influência do tratamento com AG frente a exposição aguda tipo *binge* e subcrônica de KET sobre parâmetros comportamentais de memória e locomoção em ratos adolescentes;
- Avaliar a influência do tratamento com AG sobre parâmetros bioquímicos plasmáticos além do status oxidativo hepático e renal dos animais previamente expostos a administração aguda tipo *binge* e subcrônica de KET;
- Avaliar a influência do tratamento com AG sobre o status oxidativo hipocampal induzido pela exposição aguda tipo *binge* e subcrônica de KET;
- Avaliar os níveis de imunoreatividade de marcadores moleculares (pró-BDNF, BDNF e TrKB) no hipocampo de animais previamente expostos a administração aguda tipo *binge* e subcrônica de KET e posteriormente tratados com AG.

3. REVISÃO BIBLIOGRÁFICA

3.1 CONTEXTO HISTÓRICO DA CETAMINA

A KET foi sintetizada em 1962 pelo químico americano Calvin Lee Stevens e seus colegas no laboratório *Parke & Davis*, nomeada inicialmente de "CI 581'', essa substância substituiu o uso da fenciclidina após sua característica psicotomimética ser descoberta (DOMINO, 2010). O objetivo dos pesquisadores foi produzir um composto com efeitos anestésicos semelhantes a fenciclidina, mas com menores efeitos adversos. Dessa forma, o primeiro teste clínico que usou a KET ocorreu em agosto de 1964 com uma dose sub anestésica (DOMINO, 2010). Porém, a KET também demonstrou características psicotomiméticas, mas esses efeitos adversos foram menores quando comparados aos da fenciclidina (DOMINO, 2010). Muitos pacientes descreveram sensações incomuns, como o efeito de flutuar sobre o corpo, que é semelhante a um estado cataléptico, pois este pode ser resultado da dissociação funcional e eletrofisiológica entre os sistemas límbico e o tálamo-neocortical (GARCIA, 2007; DOMINO, 2010). Diante disso, a KET foi classificada como um anestésico dissociativo (DOMINO, 2010).

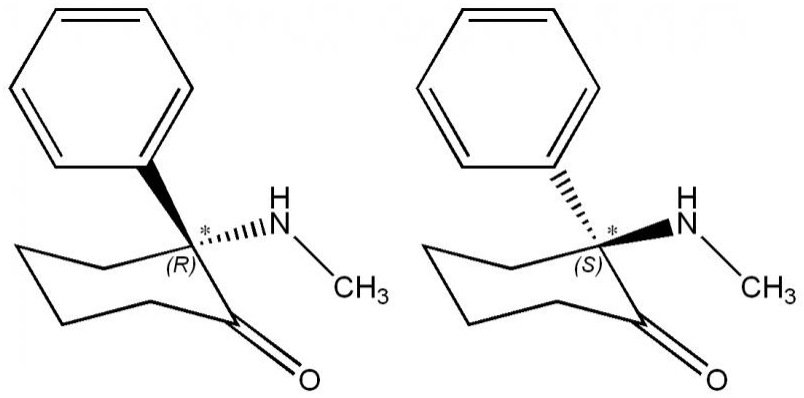
Após aprovada pela FDA, a KET foi bastante usada por soldados americanos, na Guerra do Vietnã, com finalidade anestésica (KOHRS; DURIEUX, 1998; DOMINO, 2010). No entanto, por se tratar de uma droga psicotomimética, em 1971 surgiu o primeiro caso de uso abusivo da KET nos Estados Unidos da América (SIEGEL, 1978). Com o passar dos anos, a popularidade da KET, por parte dos usuários recreacionais, cresceu ao ponto desses indivíduos a denominarem como "Special K", "Vitamina K" ou apenas "K" (DILLON; COPELAND e JANSEN, 2003; JANSEN e DARRACOT-CANKOVIC, 2001). Os principais efeitos gerados por essa droga que levam ao uso abusivo e ilícito são: euforia, distorções visuais e auditivas, sensações eróticas e a sensação de flutuar sobre o corpo (FREESE; MIOTTO e REBACK, 2002; GLABE, 2004). Após o aumento preocupante do uso ilícito da KET, a FDA classificou esse fármaco como droga de abuso na década de 1990, por tratar-se de uma droga com capacidade de aumentar a excitação elétrica do sistema límbico e do córtex cerebral, assim como o aumento do tônus simpático nessas mesmas áreas (BASCUÑANA et al., 2003). Dessa forma, a comercialização dessa substância ocorre de forma controlada em muitos países, estando muitas vezes restrita à prática clínica (MORGAN; CURRAN, 2012; BARRIOS;

PÉREZ, 2007). No Brasil, segundo o Ministério da Saúde (portaria nº 344, 1998), a aquisição dessa droga ocorre apenas com prescrição controlada do tipo C, de cor branca.

3.2 CETAMINA: FARMACOCINÉTICA E FARMACODINÂMICA

A KET possui características químicas análogas à fenciclidina e é conhecida quimicamente como 2-(o-clorofenil)-2-(metilamino)-cicloexanona ($C_{13}H_{16}CINO$). É comercialmente disponível na forma racêmica de dois enantiômeros o R (-) e o S (+) os quais possuem fórmula estrutural e química semelhantes (Fig. 1), porém com diferença no arranjo do carbono quiral conferindo assim, distintas propriedades farmacocinéticas e farmacodinâmicas (KOHRS; DURIEUX, 1998; LUFT; MENDES, 2005; FANTON; CORTOPASSI; BERNARDI, 2006).

Figura 1 - Estrutura química dos enatiômeros R (-) e S (+) de cetamina, respectivamente.



Fonte: Cetamina uma droga de abuso. (https://paginateste123.wordpress.com/caracteristicas-estruturais/ (S/D))

A KET apresenta alta biodisponibilidade independente da via de administração (intramuscular, intravenosa, nasal, oral ou retal), possui característica lipofílica, com rápida ação e tempo de meia-vida curto (2 a 3 horas) (GARCIA, 2007; VALADÃO, 2010). O metabolismo principal dessa substância é hepático e forma três metabólitos ativos: norcetamina, 5 hidroxi-cetamina e 4 hidroxi-cetamina que são conjugados e posteriormente excretados na urina. A KET também pode ser metabolizada em uma menor parte por outros tecidos, como o rim, intestino e pulmão (GARCIA, 2007; HEMELRIJCK; WHITE, 1997; DUVAL, 2004).

O mecanismo de ação da KET ocorre principalmente pelo bloqueio de forma não competitiva do receptor NMDA de glutamato, ou seja, pela característica de antagonizar o

receptor NMDA (GARCIA, 2007). No entanto, a especificidade da droga é inversamente proporcional a quantidade administrada, pois quanto menor a dose, maior o seu efeito (EIDE, et al. 1997). Existem ainda, outros receptores nos quais a KET pode ligar-se, sendo eles: receptores serotonérgicos, gabaérgicos e opióides. (GARCIA, 2007).

Os receptores NMDA são ativados pelo glutamato, o principal neurotransmissor excitatório do sistema nervoso central (SNC) presente em aproximadamente 80% dos neurônios cerebrais, tais receptores desempenham funções como a indução da plasticidade sináptica e a formação e consolidação da memória e do aprendizado (BANNERMAN et al., 1995, GONDA, 2012). Neste contexto, a KET ao antagonizar esses receptores, leva o SNC a uma hipofunção glutamatérgica e consequentemente a redução da estimulação neuronal, acarretando assim nos efeitos anestésicos (VASCONCELOS et al., 2005; RANG; DALE; RITTER, 2007).

3.3 CETAMINA E SISTEMA COGNITIVO

A KET foi comercializada por longos anos com a finalidade de intervir como uma droga monoanestésica, ou seja, com a capacidade de induzir imobilidade, perda da consciência e analgesia (GOODMAN; GILMAN; BRUNTON 2015). Os efeitos dessa droga sobre o SNC são melhores observados na sua grande parte após o uso clínico (RAEDER; STENSETH, 2000; FISHER; CODERRE; HAGEN, 2000). Doses anestésicas provocam efeitos dissociativos, o que é caracterizado por um estado cataléptico com paralisia dos movimentos, midríase, epífora e sialorreia (ORANJE et al., 2000, UHLHAAS et al., 2007; OLIVEIRA et al., 2004). Além disso, a KET induz efeitos indesejáveis, em especial no período pós-operatório conhecido por reações emergenciais, tais como: ilusões, pesadelos e sensações de flutuar sobre o corpo. (CRAVEN, 2007; DOTSON, ACKERMAN; WEST, 1995; SILVA et al. 2010).

O sistema cognitivo é um dos sistemas cerebrais mais importantes para a vida, o hipocampo, é uma pequena estrutura situada nos lobos temporais do cérebro, e é a principal região relacionada à memória (MACHADO, 2006). Muitos estudos evidenciaram que a exposição a curto e longo prazo da KET induziu danos ao SNC em áreas relacionadas ao aprendizado e memória (HUANG et al. 2012; PAULE et al. 2011; SORIANO et al. 2010; ZOU et al. 2009; SAMPAIO et al. 2018; LIU et al. 2019; KE, 2014). Segundo Duan e colaboradores (2013), a depressão sináptica hipocampal mediada por receptores D1/D5, poderia explicar o efeito da KET sobre o prejuízo na memória de animais expostos a droga. No entanto,

Cartágenes e colaboradores (2019) associaram o estresse oxidativo hipocampal como fator determinante para explicar este mesmo efeito sobre a memória.

Por outro lado, alguns estudos pré-clínicos evidenciaram que a diminuição dos níveis do fator neurotrófico derivado do cérebro (BDNF, do inglês: *brain-derived neurotrophic factor*) induzido pela exposição à KET, poderia comprometer a memória, o aprendizado e a plasticidade sináptica dos animais (ZUO et al., 2016; ZHAO et al., 2014). O BDNF é um membro da família de neurotrofinas que auxilia na sobrevivência neuronal e na neurogênese, sendo expresso em maior abundância no cérebro e distribuído principalmente no hipocampo, corpo estriado e córtex (AID et al., 2007).

Dessa forma, a memória de trabalho é um importante processo psicológico e possui um armazenamento ultrarrápido de informação, o que acontece apenas enquanto determinado trabalho ou comportamento é desempenhado (DOS SANTOS; ANDRADE; BUENO, 2004). Em estudos pré-clínicos utiliza-se o teste comportamental do labirinto em Y como modelo experimental para avaliar a memória de trabalho em roedores (CHU et al., 2012).

3.4 ESTRESSE OXIDATIVO E CETAMINA

O estresse oxidativo (EO) é um evento que ocorre quando há um desequilíbrio entre os agentes pró-oxidantes e antioxidantes. Nesta situação, observa-se que a produção de espécies reativas (ERs) excede a capacidade do sistema antioxidante, levando a danos celulares (HALLIWELL; WHITEMAN, 2004). A oxidação de biomoléculas com perda de funções biológicas e o desequilíbrio homeostático são algumas das consequências ocasionadas pelo estresse oxidativo a nível tecidual e celular. (HALLIWELL; WHITEMAN, 2004).

Estudos apontam que a exposição à KET induz morte celular, devido ao desequilíbrio do status oxidativo no cérebro, esse desequilíbrio está muito provavelmente ligado ao mecanismo da droga, envolvendo a regulação do receptor NMDA (WANG et al., 2006). A KET ao antagonizar os receptores de NMDA faz com que aumente a concentração de glutamato na fenda sináptica, que ao ser degradado, gera moléculas reativas que podem assim serem responsáveis pelo EO (SHIBUTA et al., 2015). Além disso, em estudos adicionais a KET aumentou a expressão do receptor NMDA, o que prejudicou a homeostase intracelular de cálcio e induziu o estresse oxidativo e apoptose neuronal (SHIBUTA et al., 2015). Réus e colaboradores (2017) revelaram que doses subanestésicas da KET (15 e 25 mg/ kg) em ratos *Wistar* aumentaram a peroxidação lipídica e a carbonilação de proteínas e diminuiram a

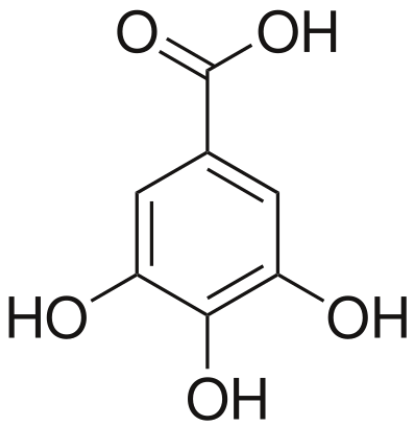
atividade da superóxido dismutase e da catalase em algumas regiões cerebrais, como o hipocampo, córtex e estriado, evidenciando assim o potencial efeito neurotóxico da droga.

De outro modo, foi elucidado os efeitos pró-oxidantes da KET em orgãos responsáveis pelo metabolismo e excreção da droga. A exposição subcrônica de KET em ratos adolescentes foi associada a um comprometimento da função hepática e renal, uma vez que essa droga aumentou os níveis séricos de aminotransferase de aspartato (AST), alanina aminotransferase (ALT), creatinina e uréia nos animais. Todos esses efeitos foram associados ao estresse oxidativo que também foi evidenciado no estudo (ONAOLAPO et al., 2019). Além disso, quando admnistrada a longo prazo, a KET provocou alterações histológicas (degenereção celular e vacuolização) e toxicidade hepatocelular dose dependente em ratos *Sprague-Dawley* (KALKAN et al., 2014). Por outro lado, a exposição da KET induziu fibrose renal e síndrome do tipo cistite, em um modelo animal de adição, no qual seses efeitos tóxicos observados, foram relacionados tanto à droga, quanto aos seus metabólitos que são excretados na urina (JANG et al., 2017).

3.5 ÁCIDO GÁLICO E SUAS PROPRIEDADES TERAPÊUTICAS

O ácido gálico (AG) ou ácido3,4,5-tri-hidroxibenzóico (Fig. 2), é um composto fenólico natural abundante no reino vegetal encontrado em plantas medicinais como o carvalho (*Quercus spp*.) e o chá-verde e em frutas (como o caju, a gabiroba e o romã) (LU et al., 2006; ROCHA, et al., 2011; FERNANDES; SALGADO, 2016). As características organolépticas do AG são: textura sólida, incolor ou moderadamente amarela. (National Institutes of Health, 2015). Sua aplicação varia da indústria alimentícia, como conservante em alimentos e bebidas, até a indústria farmacêutica para síntese de medicamentos como o trimetoprim (BAJPAI; PATIL, 2008). O AG pode ser isolado por diferentes métodos cromatográficos ou ainda pode ser sintetizado por meio da degradação hidrolítica do ácido tânico com auxílio enzimático (FERNANDES; SALGADO, 2016).

Figura 2 – Estrutura química do ácido gálico



Fonte:ASCES PE .(http://principo.org/questo-01-uerj2016.html (2012)).

As diferentes atividades biológicas e terapêuticas do AG vem sendo muito relatadas na literatura, dentre as quais podemos observar a sua capacidade de permear a barreira hematoencefálica, conferindo a essa substância uma ação neuroprotetora (MANSOURI et al., 2013) devido a sua capacidade antioxidante a nível cerebral (RECKZIEGEL et al., 2016), além de possuir ação ansiolítica (DHINGRA; CHHILLAR; GUPTA, 2012; DHINGRA et al. 2014a); anti-inflamatória (KROES et al., 1992 ; DHINGRA et al., 2014b), anticancerígena (SUBRAMANIAN et al., 2014) e antidepressiva (CHHILLAR; DHINGRA, 2012), assim como também já foi relatado que o AG atua frente ao sistema cognitivo como intensificador de memória (FARBOOD et al., 2013).

Considerado como um antioxidante natural, o AG é capaz de inibir a produção de ERs, como o ácido hipocloroso, radicais hidroxila, ânions superóxido e peróxido de hidrogênio (HANSI; STANELY, 2009; KIM, 2007; POLEWSKI; KNIAT; SLAWINSKA, 2002).
Estudos anteriores do nosso grupo de pesquisa, relataram o efeito protetor do AG frente a animais intoxicados com chumbo, no qual o composto reduziu o dano locomotor e o estresse oxidativo cerebral nos animais (RECKZIEGEL et al., 2011; RECKZIEGEL et al., 2016).

O AG parece atuar também, como agente hepatoprotetor frente a toxicidade induzida pelo paracetamol, pois observou-se em estudo pré-clínico a reversão do aumento da AST e da ALT no soro dos animais (RASOOL et al., 2010). Além disso, Ghaznavi e colaboradores (2017) também evidenciaram em estudo pré-clínico o efeito nefroprotetor do AG frente a toxicidade da gentamicina, no qual o agente reduziu os níveis de creatinina sérica, de óxido nítrico e de

outros marcadores de estresse oxidativo renal, além de aumentar as defesas antioxidantes dos animais.

Dentre os inúmeros efeitos benéficos do AG, observamos na literatura o efeito antidepressivo do AG administrado à longo prazo, frente a ratos submetidos ao protocolo de estresse crônico, onde foi observado que o AG inibiu significativamente a atividade da enzima monoamina-oxidase (MAO), responsável pela degradação de monoaminas, pois a mesma encontra-se elevada em indivíduos com depressão (CHHILLAR e DHINGRA, 2012). Além do mais é descrito na literatura que o AG mostrou uma melhora no déficit de memória em animais, após um modelo de isquemia (FARBOOD et al., 2013). Além de outro estudo conduzido por Mansouri e colaboradores (2013), o qual verificou que o AG administrado cronicamente preveniu déficits de memória e danos oxidativos após um modelo animal do tipo Alzheimer esporádico.

4. JUSTIFICATIVA

A KET é uma droga aditiva, cujo uso abusivo a curto e longo prazo têm sido preocupante, visto que foram relatados danos renais, hepáticos, alterações de memória, alterações de locomoção e desenvolvimento de quadros semelhantes ao de esquizofrenia, após o uso da droga (ONAOLAPO et al., 2019; YADAV et al., 2017; LIU et al., 2019; TRUJILLO et al., 2008). Neste contexto, é de fundamental importância a busca por agentes antioxidantes naturais que possam contribuir para redução de danos oxidativos e alterações comportamentais induzidos pelo uso ilícito e abusivo da KET. Dessa forma, a hipótese do presente estudo consiste na redução dos danos oxidativos e preservação dos padrões moleculares e comportamentais induzidos pela exposição aguda tipo *binge* (Binge-KET) e subcrônica (SbChro-KET) à droga, após tratamento com AG.

5. DESENVOLVIMENTO

5.1 MANUSCRITO CIENTÍFICO

Os resultados inseridos nesta dissertação apresentam-se sob a forma de um manuscrito científico, o qual se encontra aqui estruturado. Os itens Materiais e Métodos, Resultados, Discussão e Referências encontram-se no próprio manuscrito, seguindo as normas exigidas pelo periódico internacional, para o qual foi submetido.

Binge- and subchronic- exposure to ketamine promote memory impairments and damages in hippocampus and peripheral tissues in rats: gallic acid protective effects

Brum G. F.[a], Rosa H. Z.[a], Rossato D. R.[b], Rosa J. L. O.[b], Metz V. G.[a], Milanesi L. H.[a], Burger M.E.[ab]

[a]Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil

[b]Departamento de Fisiologia e Farmacologia, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil

Corresponding author

Higor Zuquetto Rosa (Msc.)

higor.zr@hotmail.com

Universidade Federal de Santa Maria (UFSM)

Pharmacology Graduation Program, UFSM

Santa Maria-RS, 97105-900-Brazil

Burger, M.E., M.Sc.; Ph.D.

Marilise.burger@ufsm.br

Pharmacology Post Graduation Program

Physiology and pharmacology Department

Federal University of Santa Maria (UFSM)

97105-900 Santa Maria- RS Brazil

Abstract

Ketamine (KET) is a dissociative anesthetic for restrict medical use related to high potential for abuse and neurotoxicity, what does not prevent its recreational use. Gallic acid (GA) is a natural free radical "scavenger". We evaluated the GA protective role regarding binge or subchronic (SbChro) KET-induced toxicity in adolescent rats. In the binge protocol, animals were treated with GA (one dose of 13.5 mg/kg, p.o. every 2h, totaling three doses) 12h after KET exposure (one doses of 10 mg/kg, i.p., every 3h, totaling five doses); in the SbChro, animals were treated with GA (one dose of 13.5 mg/kg/day, p.o., for 3 days) 48h following KET exposure (one dose of 10 mg/kg/day, i.p) for 10 days. Our findings show that binge-KET impaired memory, increased pro-BDNF and TrkB levels in the hippocampus, increased lipid peroxidation (LP) in kidney and hippocampus, while SbChro-KET impaired memory, increased pro-BDNF and decreased both BDNF and TrkB levels in the hippocampus, increased LP in kidney, liver and hippocampus. GA treatment better reversed the subchronically KET-induced harmful influences. Interestingly, only memory impairment observed in the SbChro-KET protocol was reversed by GA. Memory impairments showed a positive correlation with hippocampal BDNF levels and negative with LP levels in the same brain area. This last hippocampal damage (LP) showed a negative correlation with BDNF levels in hippocampus, indicating an interesting and close causal connection. Our outcomes show that the deleterious effects of SbChro-KET exposure can be attenuated or abolished with GA administration, a natural antioxidant that could be considered in KET abuse treatment.

Abbreviations: KET, ketamine; GA, gallic acid; KET+AG, ketamine + gallic acid; SbChro-KET, subchronic ketamine; Binge-KET, ketamine binge; pro-BDNF, pro–brain-derived neurotrophic factor; TrkB, Tropomyosin receptor kinase B; LP, lipid peroxidation; BDNF, brain derived neurotrophic factor; NMDA, *N-methyl-D-aspartate; FDA, food and drug administration; CNS, central nervous system; Pb, lead; AST,* aspartate aminotransferase; ALT, alanine aminotransferase; CRE, creatinine;

Keywords: Ketamine abuse. Working memory. Hepatotoxicity. Nephrotoxicity. Neurotoxicity.

1. Introduction

Ketamine (KET) is a dissociative anesthetic for restrict hospital use, whose action mechanism is based on noncompetitive blocking of the N-methyl aspartate (NMDA) type glutamate receptor [1]. Besides its anesthetic action, considered ideal for surgical procedures [2], the FDA [3] (2019) recently authorized the use of esketamine in potentially suicidal depression treatment. The KET prestige is shaken when its psychedelic and stimulating effects are evidenced, what may cause tolerance and abuse [4]. In epidemic level, KET abusive use raises concerns because among synthetic substances, its use has been considerably growing [5, 6,7, 8,]. In this sense, the FDA classifies KET as an abusive drug, leading to a controlled dispensing in many countries [5].

KET acute exposure may induce toxicity, which may be even more severe in long-term exposures [1, 2]. Preclinical studies concerning KET acute exposure have shown damages on the central nervous system (CNS), especially affecting brain areas related to learning and memory [9, 10]. However, KET frequent use can lead to a compulsive pattern because of hallucinations, paranoia, empathy, erotic dreams and feeling of floating out of body, which are all evoked by this drug [11, 12, 13, 14, 15, 16]. Furthermore, prolonged KET exposure can lead to locomotor and learning damages, since this drug has been related to decreased neurogenesis [17, 18] and it may also lead to increased free radicals generation, what consumes the endogenous antioxidant defenses and favors oxidative stress and neuroinflammation development [19, 20].

On the other hand, gallic acid (GA) (3,4,5-trihydroxybenzoic acid) is a natural phenolic compound found in fruits and teas, presenting a range of pharmacological properties, being the main ones antioxidants and anti-inflammatory property [21, 22, 23]. A previous experimental study from our research group showed the beneficial influence of nutshell aqueous extract (*Carya illinoensis*), whose major compound is GA, on oxidative damages consequent to cigarette smoke exposure, besides minimizing locomotor impairments and other abstinence symptoms [24]. In sequence of our studies, we also observed GA beneficial influences on lead (Pb) poisoning [25], when this phenolic compound was able to revert oxidative damages in brain, liver, kidneys and blood of rats, minimizing Pb-induced locomotor impairments [24, 25]. In addition, movement disorders such as reserpine-induced orofacial dyskinesia was reversed with GA, confirming its antioxidant and protective properties [26]. In fact, the protective properties attributed to GA are extensive experimental, once they have been observed on decreased hepatic toxicity induced by paracetamol and methotrexate [22,27] and they reduce

gentamicin-induced renal toxicity as well. [28]. Considering CNS, GA has also been promising, showing antioxidant properties in both stressed and non-stressed rats, decreased depression-like behaviors [29] and prevented memory deficits related to oxidative damages in a sporadic animal model of Alzheimer's [30].

Which the mentioned above, it is urgent the search for treatments with agents can reverse or minimize the damages induced by KET abusive exposure. In this sense, our aim was to evaluate the beneficial influence of a treatment with GA on oxidative, molecular and memory behavioral parameters as a result of binge-like or SbChro-KET exposure.

2. Materials and methods

2.1 Animals

Forty-five male *Wistar* rats (150±20 g) were used for this study. All animals were allocated in Plexiglas cages with filtered water and food *ad libitum*. They were kept in a room with a 12-hour light/dark cycle, with air exhaustion and controlled temperature (22±2 °C). This experiment was approved by the Animal Ethics Committee of the Federal University of Santa Maria (UFSM/8629131218) associated to the National Council for Animal Experimental Control (CONCEA**).**

2.2 Drugs and solutions

Hydrochloride ketamine (União Química Farmacêutica Nacional S/A, 10%) KET was dissolved in saline (NaCl 0,9%) up to a concentration of 10 mg/kg, while gallic acid (Neon Comercial LTDA, PM: 188,13) (GA), was dissolved in distilled water up to a concentration of 13.5 mg/kg.

2.3 Experimental protocol

Binge-like protocol (Fig. 1A): The animals were first divided in two experimental groups: control (Saline, NaCl 0.9% i.p.; n=12) and KET (10 mg/kg i.p.; n=12) groups, which received, five administrations of KET or saline, with three hours of interval between each administration, mimicking a binge-type exposure [31, 32]. After 12 hours from the last KET administration, the animals were subdivided in four groups: i) CONTROL (n=6); ii) GA (n=6); iii) KET (n=6); iv) KET+GA (n=6) where they received the treatment by a gavage (GA 13.5 mg/kg or vehicle (distilled water) being three administrations with interval of two hours between each administration [adapted from 24,25]. At the sequence of working memory and locomotion

parameters were acessed in the Y-maze task [33,34]. On the next day, rats were anesthetized with isoflurane (to the effect) and euthanized by cardiac puncture. Blood plasma, liver and kidney were collected as the brain tissue was removed and the hippocampus was dissected for further biochemical and molecular analyses.

Subchronic protocol (Fig. 1B): rats were randomly divided in two experimental groups: control (Saline, NaCl 0.9%; i.p.; n=10) and KET (13.5 mg/kg; i.p.; n=11), the animals received KET or saline, for 10 days in their respective groups. 48 hours after the last drug administration, the animals were subdivided in 4 groups: i) CONTROL (n=5), ii) GA (n=5), iii) KET (n=5) and iv) KET+GA (n=6) and received the treatment by gavage (GA 13.5 mg/kg or vehicle (distilled water) 1x/day for three days [adapted from 24,25]. On the following day, working memory and locomotion were evaluated in the Y-maze task [33,34]. 24 hours after behavioral assessments, the animals were anesthetized with isoflurane (to the effect) and euthanized by cardiac puncture. Blood plasma, liver and kidney were collected. The brain tissue was removed and the hippocampus was dissected for further biochemical and molecular analyses.

2.4 Behavioral test

2.4.2 Y-Maze test

This test evaluates the working memory of the animals (% alternation) and the locomotor activity (number of total inputs). Y-maze apparatus consisted of three arms Y-shaped according to Chu et al. (2012). The animals were placed individually in the center of the apparatus and they were evaluated during a 5-minute session. The alternation (three consecutive entries in different arms) and total number of entries in the arms were evaluated according to Chu et al., (2012) and Segat et al., (2016) and, calculated % alternation score was in accordance with to the following formula:

$$\left(\frac{\text{Total alternation number}}{\text{Total entries number} - 2}\right) 100$$

2.5 Western blotting analysis

Hippocampal tissues were homogenized with a lysis buffer (20mM Tris-HCl pH=8.0, 137 mM NaCl, 1% NP40, 10% glycerol, 10 $\mu g.mL^{-1}$aprotinin, 1mM phenylmethylsulfonylfluoride (PMSF), 0.5 mM sodium vanadate, 0.1 mM benzethonium chloride). Homogenates were centrifuged to obtain supernatants where total protein concentration was determined according

to the MicroBCA procedure (Pierce, IL, USA), using bovine serum albumin as standard. Briefly, the separation of protein samples were carried out by electrophoresis on a 12.5% polyacrylamide gel (according to protein molecular weight) and electrotransferred to a PVDF membrane (Millipore, MA, USA). Non-specific binding sites were blocked in Tris-buffered saline (TBS), pH 7.6, containing 5% non-fat dry milk. Membranes were rinsed in buffer (0.05% Tween 20 in TBS) and incubated with primary antibodies: anti-actin (1:20000), anti-BDNF (1:1000; Abcam, Cambridge, UK), anti-proBDNF (1:1000; Abcam, Cambridge, UK), anti-TrkB (1:500; Santa Cruz Biotechnology, CA, USA) followed by anti-rabbit IgG horseradish peroxidase conjugate (1:20000; Santa Cruz Biotechnology). The immune complexes were visualized by chemiluminescence using the ECL kit (Amersham Pharmacia Biotech Inc., NJ, USA) according to manufacturer's instructions. The film signals were digitally scanned and quantified using ImageJ® software. Actin was used as the internal control for Western blot and the values were used for experimental data standardization.

2.6 Biochemical assays

2.6.1 Plasma analysis

The dosages of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine (CRE) were performed using a commercial kit (Labtest®). All tests were performed in one BIO-2000 IL Semi-Automatic Biochemical Analyzer.

2.6.2 Lipid peroxidation (LP)

Lipid peroxidation was evaluated in the liver, kidney and hippocampus of rats, expressed in nmol malondialdehyde (MDA / g) tissue and described in detail by Ohkawa, Ohishi and Yagi [35]. The pink staining is quantified by a colorimetric assay at 532 nm wavelength.

2.6.3 Catalase (CAT) activity

CAT activity was performed in the liver, kidney and hippocampus. This technique evaluates the H_2O_2 degradation, for 120 s, with CAT activity expressed in μmol H_2O_2/min/g tissue. The applied protocol is in accordance with Aebi [36].

2.7 Statistical analysis

The differences among the four experimental groups were analyzed by two-way ANOVA, followed by the Duncan's test and Pearson's correlations was realized, also (Statistica

8.0 software package for Windows was used). Values of p <0.05 were considered statistically significant for all comparisons. GraphPad Prism® (version 5.01) was used to design the figures.

3. Results

3.1 Binge-protocol

3.1.1 Gallic acid (GA) influence on working memory in binge-ketamine (KET) exposed animals in the Y-maze paradigm (Figure 2)

Two-way ANOVA revealed a drug main significant effect [F(1,20)=35.71, *P*=0.00] on the percentage of alternations in the Y-maze task. The binge-like exposure to KET decreased de percentage alternation in this behavioral paradigm, independently of the GA treatment (KET+GA group), whose values were smaller when compared to saline and GA groups, respectively (Fig. 2A). Binge-KET or GA treatment exerted no influences on entries total number, indicating no locomotion impairments in this behavioral paradigm (Fig. 2B).

3.1.2 Gallic acid (GA) influence on plasma biochemical markers from plasma of binge-ketamine (KET) exposed animals (Table 1)

Two-way ANOVA revealed a drug main significant effect, treatment and drug x treatment interaction [F(1,20)=33.61, *Pb* <0.000; 32.22, *P*<0.000 and 5.89, *P*=0.025, respectively] on creatinine plasma levels; a treatment main effect [F(1,20)= 6.42, *P*<0.019] on ALT besides drug and treatment [F(1,20)= 5.46, *P*<0.029 and 14.53, *P*<0.001, respectively] on AST plasma levels.

KET binge exposed animals showed increased creatinine plasma levels in relation to the control group, while the KET+GA group showed a partial recovery of this level, whose values were similar to control group, but different from the GA group. ALT and AST levels were not modified by KET exposure, but the KET+GA group showed increased level of both ALT and AST in relation to KET group (Table 1).

3.1.3 Gallic acid (GA) influence on the oxidative markers (lipid peroxidation levels- LP and catalase activity - CAT) in liver, kidney and hippocampus of binge-ketamine (KET) exposed animals (Table 2)

In the liver, two-way ANOVA revealed a significant main effect of the treatment [F(1,20)= 32.67, *P*<0.000] on LP levels, and drug and treatment [F(1,20)= 20.11, *P*<0.000 and 24.10, *P*<0.000, respectively] on CAT activity.

In the kidney, two-way ANOVA revealed a drug main significant effect and treatment [F(1,20)= 8.01, *P*<0.01 and 18.01, *P*<0.000, respectively] on LP levels, and drug [F(1,20)= 80.72, *P*<0.000] on CAT activity.

KET binge exposed group showed increased LP levels in the kidney in comparison to control group, while this oxidative marker did not change in the liver. The KET+GA group showed reversion of LP levels in the kidney, while a decreasing was observed in the liver, in comparison to KET group (Table 2).

Binge-KET exposure increased CAT activity in the liver and decreased in the kidney, in comparison to the control group. The KET+GA group showed a partial reversion of this activity in the liver, whose values were smaller than KET, but higher than GA-group. In the kidney both KET and KET+GA groups presented similar CAT activity (Table 2).

In the hippocampus, two-way ANOVA revealed a drug main significant effect [F(1,20)= 11.73, *P*=0.002] on LP levels, and drug and treatment [F(1,20)= 56.88, *P*<0.000 and 17.36, *P*<0.000, respectively] on CAT activity.

Binge-KET exposed group showed increased LP levels and CAT activity in relation to control group in the hippocampus. While the increased LP levels was not recovered by GA treatment (KET+GA group), CAT activity was partially reversed by GA treatment (KET+GA group), whose values were smaller than KET but higher than GA alone (GA-group) (Table 2).

3.1.4 Gallic acid (GA) influence on the molecular markers (pro-BDNF, BDNF and TrkB immunoreactivity) in the hippocampus of binge-ketamine (KET) exposed animals (Figure 3)

In the hippocampus, two-way ANOVA revealed a drug main significant effect, treatment and drug x treatment interaction [F(1,20)= 14.29, *P=0.001,* 4.91, *P=0.038 and* 70.31, *P=0.000, respectively*]. On pro-BDNF, [F(1,20)= 8.88, *P=0.007,* 9.03, *P=0.006 and* 15.89, *P=0.000, respectively*] on BDNF and a drug main significant effect and drug x treatment interaction [F(1,20)= 369.27, *P=0.000 and* 32.51, *P=0.000, respectively*] on TrkB levels.

While GA treatment increased *per se* pro-BDNF, BDNF and TrkB immunoreactivity, binge-KET exposure was able to increase both pro-BDNF and TrkB in comparison to the control group (Fig. 3A, 3B and 3C). In fact, the GA treatment (KET+GA group) was able to

reverse the immunoreactivity of both pro-BDNF and TrkB, whose levels were smaller than the ones observed in the KET group (Fig. 3A and 3C).

3.2 Subchronic ketamine (SbChro-KET) protocol

3.2.1 Gallic acid (GA) influence on working memory in animals sub-chronically exposed to ketamine, which was observed in Y-maze paradigm (Figure 2)

Two-way ANOVA revealed a drug main significant effect and drug x treatment interaction [$F(1,20)=6.33$, $P=0.022$ and 10.52, $P=0.005$, respectively] on the percentage of alternations in the Y-maze task. SbChro-KET decreased this memory parameter, which was reversed in the KET+GA group. In fact, KET+GA showed similar percentage of alternations in the Y-maze task than GA group, what indicates comparable working memory between them (Fig. 2C). SbChro-KET exposure and GA treatment did not affect the locomotion index, as observed in similar total arms entries in the Y-maze task (Fig. 2D)

3.2.2 Gallic acid (GA) influence on plasma biochemical markers in plasma of animals subchronically exposed to ketamine (Table 1)

Two-way ANOVA revealed a drug main significant effect and drug x treatment interaction [$F(1,20)= 30.65$, $P<0.000$ and 15.81, $P<0.000$, respectively] on ALT, and a significant drug main effect [$F(1,20)= 24.63$, $P<0.000$] on AST plasma levels as well.

While KET exerted no influence on creatinine levels, ALT and AST plasma levels were increased by this SbChro-KET exposure in comparison to the control group. In fact, KET+GA group showed total reversion of ALT levels, whereas AST was partially reversed, since this level was smaller than those observed in the

KET-, but higher that those observed in the GA-group (Table 1).

3.2.3 Gallic acid (GA) influence on the oxidative markers (lipid peroxidation levels-LP and catalase activity-CAT) in liver, kidney and hippocampus of animals sub-chronically exposed to ketamine (Table 2)

In the liver, two-way ANOVA revealed a significant main effect of the treatment [$F(1,20)= 32.67$, $P<0.000$] on LP levels. In the kidney, two-way ANOVA revealed a drug main significant effect, treatment and drug x treatment interaction [$F(1,20)= 11.66$, $P=0.003$ and 4.64, $P=0.045$ and 7.76, $P=0.012$, respectively] on LP levels, and drug x treatment interaction [$F(1,20)= 8.28$, $P=0.010$] on CAT activity.

KET exposed group showed increased LP levels in both liver and kidney in relation to the control group, while the KET+GA exposed animals showed inversed response. In addition, increased CAT activity was observed in both liver and kidney, which were reversed in the KET+GA groups (Table 2).

In the hippocampus, two-way ANOVA revealed a drug main significant effect, treatment and drug x treatment interaction [F(1,20)= 7.07, *P*<0.016; 7.58, *P*<0.013; 14.57, *P*<0.001, respectively] on LP levels.

SbChro-KET exposure increased the hippocampus level of LP in relation to control group, while the GA treatment (KET+GA group) totally recovered this effect, since this value was smaller than those observed in the KET treatment. CAT activity was not modified by KET, in the SbChro-KET exposure in this brain area, when all the experimental groups showed similar CAT activity regardless of the treatment (Table 2).

3.2.4 Gallic acid (GA) influence on the molecular markers (pro-BDNF, BDNF and TrkB immunoreactivity) in the hippocampus of animals sub-chronically exposed to ketamine (Figure 3)

In the hippocampus, two-way ANOVA revealed a drug main significant effect and drug x treatment interaction [F(1,20)= 276.62, *P=0.000* and 33.47, *P=0.000,* respectively]. On pro-BDNF, [F(1,20)= 86.61, *P=0.000 and* 16.79, *P=0.000,* respectively] on BDNF and a drug main significant effect, treatment and drug x treatment interaction [F(1,20)= 74.55, *P=0.000*, 5.89, *P=0.024* and 183.37, *P=0.000* respectively] on TrkB levels.

While GA treatment increased pro-BDNF, thus decreasing both BDNF and TrkB immunoreactivity *per se,* in the hippocampus SbChro-KET increased pro-BDNF, decreasing both BDNF and TrkB levels in comparison to the control group (Fig. 3D, 3E and 3F). In fact, levels of all these molecular markers were reversed by GA treatment. (Fig. 3D, 3E and 3F).

3.2.5 Pearson's correlations

Significant correlations were observed between behavioral and biochemical parameters from the SbChro-KET exposure: KET-induced impairments in working memory, which were observed by the percentage of alternations in the Y-maze task showed significant negative correlation with LP levels in the hippocampus (r^2= 0.40; *P*= 0.002; Fig. 4A). BDNF levels in the hippocampus showed positive correlation with working memory (observed by % alternation

in the Y-maze task) (r^2= 0.40; *P*= 0.002; Fig. 4B) and negative correlation with LP levels in the hippocampus (r^2= 0.28; *P*= 0.012; Fig. 4C).

4. Discussion

In this study, we evaluated different toxicological aspects induced by abusive exposure to KET: excessive acute (binge) and abusive sub-chronic (SbChro), quantified by biochemical and molecular analyses, besides behavioral observation, which allowed us to evidence interesting gallic acid protective properties (GA, (3,4,5-trihydroxybenzoic acid). In the first experimental protocol, binge-KET exposure caused: i) increased creatinine plasma level; ii) increased lipid peroxidation (LP) in the kidneys and hippocampus, and increased catalase (CAT) activity in liver and hippocampus and decreased CAT in the kidneys; iii) increased Pro-BDNF and TrkB impairments were not benefited by GA treatment. This antioxidant agent partially reverted all other binge-KET induced biochemical and molecular parameters, completely reversing the LP in the kidneys and hippocampus. In the second experimental protocol, SbChro-KET exposure caused: i) increased ALT and AST plasma level; ii) increased LP level in liver, kidneys and hippocampus together with increased CAT activity in both liver and kidneys; iii) increased Pro-BDNF and decreased BDNF and TrkB immunocontent in the hippocampus; iv) memory impairments.

KET is an arylcycloalkylamine: 2-(o-chlorophenyl)-2-(methylamino)-cyclohexanone that following administration, undergoes oxidation by hepatic microsomal enzymes, thus producing three active metabolites: norcetamine, 5OH-ketamine and 4OH- ketamine (Oliveira et al., 2004). All these metabolites are subsequently hydroxylated and conjugated, generating water-soluble glucuronides derivatives, which are excreted in the urine. In addition to the hepatic biotransformation, in a minor extent, KET is metabolized in the kidneys and brain as well (Garcia, 2007; Hemelrijck and White, 1997). Regarding these KET characteristics, the liver, kidneys and brain are important tissues for KET detoxification and their functionality needs to be preserved.

Teenagers have recreationally used Ketamine especially in parties, configuring an illicit, acute and compulsive use. This eventual KET use is frequently related to acute poisoning, mainly characterized by confusion, dizziness, transient period of consciousness loss, besides cardiovascular imbalances such as tachycardia and hypertension (Yiu-Cheung, 2012).

Considering this acute toxicity consequent to recreational profile and eventual ketamine use, in our first experimental protocol we observed that binge-KET exposed animals presented increased LP and decreased CAT activity in the kidneys besides increased plasma levels of creatinine, which together may be indicative of impaired renal function. Interestingly, this acute exposure was not related to lipoperoxidation in the liver, what can be explained by the increased CAT activity, which apparently managed lipoperoxidation. Considering the GA treatment, while this antioxidant compound was able to reverse the renal LP induced by binge-KET, creatinine plasma level was not totally recovered by GA, indicating that part of the drug-induced toxicity remains. From these findings, we can infer that binge-KET exposure may be related to excessive drug serum level and its derivatives, overcoming liver clearance, consequently requiring renal metabolism, what may have favored the impairments development in this tissue, as it was observed in this study. This interpretation is in agreement with literature findings, where it has been shown that KET can lead to renal failure, since its acute administration may exerts a direct toxic effect on the urinary tract (Chu et al., 2007).

Besides its acute use, KET has now become a recognized abusive drug in different parts of the world, whose prolonged use may cause damages in vital organs and nervous system (Jang et al., 2017; Kalkan et al., 2014; Yadav et al., 2017), also favoring addiction development (Jang et al., 2017). In this sense, from our second experimental protocol, animals exposed to SbChro-KET showed impaired hepatic function, as observed by increased plasma AST/ALT levels together with increased LP levels and CAT activity in the liver and kidneys. Based on these findings, we can infer that SbChro-KET exposure favored reactive species generation, what is compatible with oxidative damage development and functional changes in both liver and kidneys, as it was already observed (Onaolapo, 2019). In fact, increased LP, ALT and AST are associated with impaired hepatic function following SbChro-KET administration in rats (Kalkan et al., 2014; Onaolapo, 2019). On the other hand, GA, which is a triphenolic natural compound recognized by its antioxidant potential (Punithavathi et al., 2011; Reckziegel et al., 2016), with only three doses in sequence, was able to reverse ALT and AST plasma levels, also reducing oxidative damage markers subchronically induced by KET exposure in both liver and kidneys, as observed here. In this sense, from two experimental protocols here developed with binge-acute- and sub-chronic- KET exposure, we may propose that the GA treatment was effective to minimize peripheral damages caused by this drug, especially in the SbChro-KET exposure.

The brain is also responsible for KET detoxification (Garcia, 2007; Hemelrijck and White, 1997), being also sensitive to oxidative damage induced by this drug, what is directly related to brain damages onset (Bouayed, 2009; NG, 2008). However, brain-level KET also promotes glutamate accumulation in the synaptic cleft due to the blocking of glutamatergic receptor (Dong, 2009; Maeng, 2007; Tzingounis, 2007), and this consequent glutamate accumulation, lead to cellular alterations with consequent increase of the reactive species and this way linked to cell death due to excitotoxicity (Xu and Lipsky, 2015; Du Bois, 2007). Glutamatergic projections are found in different brain areas, including hippocampus, which is a region that is closely involved in memory regulation (Khakpai et al., 2012), characterizing an important brain area for our study. In this context, from our observations, binge-KET exposure increased lipoperoxidation and CAT activity in the hippocampus, while the subchronic exposure only increased the lipoperoxidation in the same brain area, without affecting CAT activity. Our findings are consistent with literature data, which showed that KET is able to alter the brain antioxidant status in rats (Da Silva et al. 2017; Onaolapo, 2017; Schimites et al., 2019; Vasconcelos et al., 2015), while its intermittent experimental administration resulted in oxidative damages to the hippocampus (Cartágenes, 2019). In the current study only three doses of GA were enough to partially reestablish the CAT activity in hippocampus of binge-KET exposed animals, exerting no protection or recover on KET-induced hippocampal lipoperoxidation, what confirms why GA has been described as an excellent free radical "scavenger" (Isuzugawa et al., 2001). Inversely, hippocampal lipoperoxidation subchronically induced by KET was completely reversed by GA treatment, which besides being an efficient antioxidant; it has been recognized by its neuroprotective- and anti-inflammatory- property (Sarkaki, 2015). Based on this, we can hypothesize that GA treatment exerts beneficial influences against oxidative KET-induced damages in the hippocampus, proving its protective role in the neurotoxicity attenuation related to oxidative damages induced by this addictive drug (Chandrasekhar et al., 2018; Schimites et al., 2019).

The hippocampus exerts several functions, especially developing and evoking memories and/or inducing the rest of the brain cortex to do the same and due to this, the hippocampus and its connections are pivotal for this function (Izquierdo, 2007). In this sense, KET mechanism of action may be quite involved with this brain area, culminating in neuronal degeneration together with memory- and cognitive- impairments (Jevtovic-Todarovic et al., 2013; Onaolapo et al., 2017; Wang et al., 2010). Based on these considerations, our current findings showed significant changes in the hippocampal drug-induced neurotrophic factors, since increased pro-

BDNF and TrkB-R immunoreactivity were observed in binge-KET exposed animals. Pro-BDNF and its mature derivative BDNF have distinct and sometimes opposite functions, since drugs potentially addictive, including KET, are able to increase the proteolytic cleavage, converting pro-BDNF to BDNF (Lu, 2005), besides increases TrkB phosphorylation (Kohtala, 2019). Our findings are in accordance with literature data because an acute binge-KET exposure did not increase the BDNF levels, but increased its pro-BDNF precursor, as it was already shown after only a single KET administration (Nguyen, 2016). According literature, pro-BDNF induces neuronal apoptosis and atrophy, while TrkB activation has been associated with neuronal growth and survival (Lu et al., 2005). Thus, we can suggest that increased TrkB level may be a rapid compensatory response to the pro-BDNF increase aiming to maintain the neuronal homeostasis.

BDNF is the most widely and abundantly expressed neurotrophin in the brain, where it plays an important role in maintenance, survival and neuronal plasticity (Harte et al., 2007; Maya, 2018). Regarding our SbChro-KET protocol, pro-BDNF immunoreactivity remained increased, unlike TrkB and BDNF decreased their immunoreactivity. Several studies have shown that repeated (subchronic) doses of KET reduce BDNF levels in the brain (Ke, 2014; Zhao, 2014; Zuo, 2016), but specifically in hippocampus and cortex (Zuo, 2016). Our findings are in agreement with these ones, since following continued exposure to KET, a reduced pro-BDNF cleavage for BDNF was observed (Zhao, 2014), thus increasing the pro-BDNF immunoreactivity, what is not reflected on the BDNF final level. It has been well described that reduced levels of BDNF probably make neurons more vulnerable to damage and even to cell death (Hansen et al., 2004) what is in line with our outcomes related to oxidative damages in the hippocampus.

In the current study, it was possible to evidence increased of the synaptic plasticity in hippocampus after only three doses of GA, especially observed on the partial reversion of both pro-BDNF and TrkB levels binge-KET-induced, also evidenced in the sub-chronic protocol, when pro-BDNF and both BDNF and TrkB levels were partially recovered by GA treatment. According literature data, GA was able to increase hippocampal BDNF and TrkB levels (Chandrasekhar et al., 2018; Zhu, 2019), which is in agreement with our findings. This way, with the current study, we confirmed the already presented GA neuroprotective property (Chandrasekhar et al., 2018).

In fact, hippocampus is a pivotal brain area involved in anxiety and depression behaviors, also mediating memory formation and consolidation, therefore, drugs that promote

hippocampal damages are capable of inducing memory deficits (Patki, 2013). Our findings are compatible with literature data, since our two experimental paradigms, i.e. both binge- and SbChro- KET exposure were related to impaired working memory in the animals. Additionally, studies have shown that KET is able to impair short-term memory (Cartágenes, 2019; Vasconcelos, 2015) and working memory (Onaolapo, 2019) in adolescent rats. Such consequence may be related to the fact that KET, besides blocking NMDA receptors leading to oxidative damage, can activate cholinesterases, consequently decreasing acetylcholine levels and impairing memory formation at hippocampus level (Keilhoff et al., 2004; Scheller et al., 1996). Our experimental results allowed us to observe that the KET-induced working memory impairment was fully recovered by GA in the sub-chronic protocol, but this beneficial effect was not observed when KET exposure was binge-acute. Studies have shown that the GA treatment significantly improved memory impairments induced by different substances (Corona et al., 2013; Mansouri, 2013; Sarkaki, 2015). In most of these studies, authors linked better memory performance with GA free radicals "scavenger" property (Deshmukh et al., 2009; Hansi and Stanely, 2009; Polewski et al., 2002). In this sense, our current findings are in agreement with literature, considering the GA beneficial influence on the memory, since we show for the first time that the GA treatment for only three days, subsequent to sub-chronic exposure to KET, was enough to reverse memory damages. Our protocol of acute binge-KET exposure, due to the excessive exposure to drug in a short period, allowed the drug hepatic metabolism to be extrapolated to other tissues, such as kidneys and brain, provoking damages in these tissues, as observed here. It is important to highlight that both oxidative and molecular damages observed in the hippocampus could give basis to a hypothesis that these damages are directly related to the memory impairment observed in this study. In fact, these oxidative and molecular damages were provoked in the acute binge-KET exposure, making difficult the GA protective influences, disfavoring memory performance, as observed in the behavioral test. Inversely to acute KET, SbChro-KET exposure protocol, the use of a small drug amount for a longer period allowed a broader hepatic metabolism, reducing damages, especially in the evaluated brain tissue.

In comparison with the acute protocol, subchronically KET-induced impairments were more clearly reversed by GA treatment, since ALT plasma- and LP-levels in liver, kidney and hippocampus, and CAT activity in both liver and kidney were completely reversed by the GA treatment. Only a partial reversion was observed in the AST plasma level and hippocampal Pro-

BDNF, BDNF and TrkB immunoreactivity, which together, culminated in a complete memory recovery, as observed through significant correlations shown in this study.

5. Conclusion

In summary, for the first time we show the GA protective role on peripheral and brain tissues, comparing two different exposures to KET: in the first, animals were acutely exposed to the drug (binge-KET), while in the second, animals were subchronically exposed to the drug (SbChro-KET). Treatment with GA after binge-KET exerted some protection on kidneys and brain (hippocampus) without recovering the impaired working memory. In the sub-chronic KET exposure, GA exerted protection on oxidative markers in the liver, kidneys and hippocampus, reversed molecular impairments and recovered the impaired memory performance. Considering our findings, we suggest that GA could be, after further toxicity studies, an alternative treatment for acute poisoning and for KET chronic use. Additional studies involving mechanisms of reward, which are related to addiction development should be conducted with KET and GA.

Declaration of Competing Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Acknowledgement

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. The funding had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Fundação de Amparo à Pesquisa do estado do Rio Grande do Sul (FAPERGS) and Pró-reitoria de Pós-Graduação e Pesquisa (PRPGP- PROAP/UFSM).

References

[1] J.B.S Garcia. Cetamina uma nova leitura. Prática Hospitalar. 53, (2007) 214-6.

[2] M.G. Annetta, D. Iemma, C. Garisto, C. Tafani, R. Proietti. Ketamine: New indications for an old drug. Current Drug Dargets. 6 (2005). 789-794.

[3] Food and Drug Administration U.S., FDA approves new nasal spray medication for treatment-resistant depression; available only at a certified doctor's office or clinic, 2019. https://www.fda.gov/news-events/press-announcements/fda-approves-new-nasal-spray-medication-treatment-resistant-depression-available-only-certified. [access on july 18, 2019].

[4] C.J Morgan, H.V. Curran. Independent Scientific Committee on Drugs (ISCD). Ketamine use: a review. Addiction. 107 (2012) 27-38.

[5] F.C. Silva, R.T. Dantas, M.C. Citó, M.I. Silva, S.M. Vasconcelos, M.M. Fonteles, F.C.F. SOUSA. Ketamina, da anestesia ao uso abusivo: artigo de revisão. Revista Neurociências, 18 (2010) 227-37.

[6] A.Y. Onaolapo, O.J. Ayeni, M.O. Ogundeji, A. Ajao, O.J. Onaolapo, A.R. Owolabi. Subchronic ketamine alters behaviour, metabolic indices and brain morphology in adolescent rats: Involvement of oxidative stress, glutamate toxicity and caspase-3-mediated apoptosis. Journal of Chemical Neuroanatomy. 96 (2019) 22-33. DOI:10.1016/j.jchem neu.2018.12.002.

[7] World Drug Report (WDR). United Nations publication, Sales No. E.18.XI.9, 2018. [access on july 18, 2019].

[8] United Nations office on Drugs and Crime, Austria (UNODC). Annual Report. Vienna, 2014. [access on july 18, 2019].

[9] T.B. Sampaio, L.F. de Oliveira, L.C. Constantino, A.P. Costa, G.G. Poluceno, W.C. Martins, C.I. Tasca. Long-Term neurobehavioral consequences of a single ketamine neonatal exposure in rats: effects on cellular viability and glutamate transport in frontal cortex and hippocampus. Neurotoxicity Research. 34 (2018) 649-659. DOI:10.1007/s12640-018-9927-x.

[10] Y. Liu, A.Q. Li, W. Ma, Y.B. Gao, L.Q. Deng, C. Zhang, J.H. Meng. Limb remote ischemic preconditioning reduces repeated ketamine exposure-induced adverse effects in the developing brain of rats. Journal of Molecular Neuroscience. 68 (2019)58-65. DOI: 10.1007/s12031-019-01282-3.

[11] K.A. Trujillo, J.J. Zamora, K.P. Warmoth. Increased response to ketamine following treatment at long intervals: implications for intermittent use. Biological Psychiatry. 63 (2008) 178-83.

[12] J.J. Aguaviva, R. Cuartero, J. L. Martínez, L.C. Redondo, T. Río, B. Foncillas, B. Drogas de diseño: un Nuevo paciente crítico. Manejo de las intoxicaciones por drogas consumidas en macrofiestas. Salud Rural. 20 (2003) 1-9.

[13] P. Dillon, J. Copeland, K. Jansen. Patterns of use and harms associated with non-medical ketamine use. Drug and Alcohol Dependence. 69 (2003) 23-28 DOI:10.1016/s0376-8716(02)00243-0.

[14] K. L. Jansen, R. Darracot-Cankovic. The nonmedical use of ketamine, part two: A review of problem use and dependence. Journal Psychoactive Drugs. 33 (2001) 151–8. DOI 10.1080/02791072.2001.10400480.

[15] T.E. Freese, K. Miotto, C.J. Reback. The effects and consequences of selected club drugs. Journal of Substance Abuse Treatment. 23 (2002) 151-6, 2002. DOI:10.1016/S0740-5472(02)00267-2.

[16] R. S. Glabe. Acute toxic effects of club drugs. Journal of Psychoactive Drugs. 36 (2004) 303- 13. DOI:10.1080/02791072.2004.10400031.

[17] L. Huang, Y. Liu, W. Jin, X, Z. Dong. Ketamine potentiates hippocampal neurodegeneration and persistent learning and memory impairment through the PKCγ–ERK signaling pathway in the developing brain. Brain Research. 1476 (2012) 164-171. DOI :10.1016/j.brainres.2012.07.059.

[18] C.A. Crawford, A.E. Moran, T.J. Baum, M.G. Apodaca, N. R. Montejano, G.I. Park, S.A. Mcdougall. Effects of monoamine depletion on the ketamine-induced locomotor activity of preweanling, adolescent, and adult rats: sex and age differences. Behavioural Brain Research, (2019). DOI: 10.1016/ j.bbr. 2019 .1 12267.

[19] Y. Li, R. Shen, G. Wen, R. Ding, A. Du, J. Zhou, Z. Dong, X. Ren, H. Yao, R. ZHAO, G. Zhang, Y. Lu, X. Wu. EffectS of ketamine on levels of inflammatory cytokines IL-6, IL-1β, and TNF-α in the hippocampus of mice following acute or chronic administration. Frontiers in pharmacology. 8 (2017) 139. DOI:10.3389/fphar.2017.00 139.

[20] M. Yadav, D.K. Jindal, M.S. Dhingra, A. Kumar, M. Parle, S. Dhingra. Protective effect of gallic acid in experimental model of ketamine-induced psychosis: possible behaviour, biochemical, neurochemical and cellular alterations. Inflammophar macology. 26 (2017) 413-424. DOI:10.1007/s10787-017-0366-8.

[21] A. Sarkaki, Y. Farbood, M. K. Gharib-Naseri, M. Badavi, M. T. Mansouri, A. Haghparast, M. A. Mirshekar. Gallic acid improved behavior, brain electrophysiology, and inflammation in a rat model of traumatic brain injury. Canadian journal of physiology and pharmacology. 93 (2015) 687-694.

[22] F. Safaei, S. Mehrzadi, H. Khadem Haghighian, A. Hosseinzadeh, A. Nesari, M. Dolatshahi, M. Goudarzi. Protective effects of gallic acid against methotrexate-induced toxicity in rats. Acta Chirurgica Belgica, 118 (2018) 152-160.

[23] M. Daglia, A. Di Lorenzo, F. S. Nabavi, S. Z. Talas, M.S. Nabavi. Polyphenols: well beyond the antioxidant capacity: gallic acid and related compounds as neuroprotective agents: you are what you eat. Current Pharmaceutical Biotechnology. 15 (2014) 362–372. DOI: 10.2174/1389201015041408251207 37.

[24] P. Reckziegel, V.T. Dias, D. Benvegnú, N. boufleur, R.C. Silva Barcelos, H.J. Segat, C.S. Pase, C.M. Dos santos, E.M. Flores, M.E. Bürger. Locomotor damage and brain oxidative

stress induced by lead exposure are attenuated by gallic acid treatment. Toxicology. Letters. 203 (2011) 74–81. DOI:10.1016/j.toxlet.2011.03.006.

[25] P. Reckziegel, V.T. Dias, D. Benvegnú, N. Boufleur, R.C. Silva Barcelos, H.J. Segat, C.S. Pase, C.M. Dos Santos, E.M. Flores, M.E. Bürger. Antioxidant protection of gallic acid against toxicity induced by Pb in blood, liver and kidney of rats. Toxicology Reports. 3 (2016) 351-356.

[26] P. Reckziegel, L.R. Peroza, L.F. Schaffer, M.C. FerrarI, C. M. DE Freitas, M. E. Bürger, R. Fachinetto. Gallic acid decreases vacuous chewing movements induced by reserpine in rats. Pharmacology Biochemistry and Behavior. 104 (2013) 132-137. DOI: 10.1016/j.pbb.2013.01.001.

[27] M. K. Rasool, E.P. Sabina, S.R. Ramya, P. Preety, S. Patel, N. Mandal, P.P. MISHRA, J. Samuel. Hepatoprotective and antioxidant effects of gallic acid in paracetamol-induced liver damage in mice. Journal of Pharmacy and Pharmacology. 62 (2010) 638–643. DOI:10.1211/jpp.62.05.0012.

[28] H. Ghaznavi, I. Fatemi, H. Kalantari, S.M.T. Hosseini Tabatabaei, M. Mehrabani, B. Gholamine, M. Kalantar, S. Mehrzadi, M. Goudarzi. Ameliorative effects of gallic acid on gentamicin-induced nephrotoxicity in rats. Journal of Asian natural products research. 20 (2017) 1182-1193. DOI:10.1080/1028 6020.2017.1384819.

[29] R. Chhillar, D. Dhingra. Antidepressant-like activity of gallic acid in mice subjected to unpredictable chronic mild stress. Fundamental & Clinical Pharmacology. 27. n. (2012) 409-18. DOI:10.1111/j.1472-8206.2012.01040.x.

[30] M.T. Mansouri, B. Naghizadeh, B. Ghorbanzadeh, Y. Farbood, A. Sarkaki, K. Bavarsad. Neuroprotective effects of oral gallic acid against oxidative stress induced by 6-hydroxydopamine in rats. Food Chemistry. 138 (2013) 1028-1033. DOI:10.1016/j. foodchem.2012.11.022.

[31] J.A. Clements, W. S. Nimmo. Pharmacokinetics and analgesic effect of ketamine in man. British Journal of Anaesthesia. 53 (1981) 27-30. DOI:10.1093/bja /53.1.27.

[32] J. Idvall, I. Ahlgren, K.F. Aronsen, P. Stenberg. Ketamine infusions: pharmacokinetics and clinical effects. British Journal of Anaesthesia. 51 (1979) 1167-1173. DOI:10 .1093/bja/51.12.1167.

[33] J. Chu, P.F. Giannopoulos, C. Ceballos-Diaz, T.E. Golde, D. Pratico. Adeno-associated virus-mediated brain delivery of 5-lipoxygenase modulates the AD-like phenotype of APP mice. Molecular neurodegeneration. 7 (2012) 1. DOI 10.1186/1750-1326-7-1.

[34] H.J. Segat, F. Martini, R.C.S. Barcelos, C.A. Brüning, C.W. Nogueira, M.E. Burger. m-Trifluoromethyl-diphenyldiselenide as a pharmacological tool to treat preference symptoms related to AMPH-induced dependence in rats. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 66 (2016) 1-7. DOI:10.1016 /j.pnpbp.2015.11.002.

[35] H. Ohkawa, N. Ohishi, K. Yagi. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry. 95 (1979) 351-358, https://doi .org/10.1016/0003-2697(79)90738-3.

[36] H. Aebi. Catalase in vitro. Methods in Enzymology. 105 (1984) 121–126, https://doi.org/10.1016/S0076-6879(84)05016-3.

[37] A. H. Dickenson, J. Ghandehari. Anti-convulsantsand anti-depressants. Handb. Experimental Pharmacology. 177 (2007) 145–177.

[38] C.M.B.D Oliveira, R.K. Sakata, A.M. Issy, J.B.S. Garcia. Cetamina e analgesia preemptiva. Revista Brasileira de Anestesiologia. 54 (2004) 739-52, http://dx.doi.org/10.1590/S0034-70942004000500016.

[39] J.V. Hemelrijck, P. White. Nonopioid Intravenous Anesthesia. In: Barash PG, Cullen BF, Stoelting RK (eds.). Clinical Anesthesia. 3th ed. Philadelphia: Lippincott-Raven. (1997) p.312-27.

[40] C. Yiu-Cheung. Acute and chronic toxicity pattern in ketamine abusers in Hong Kong. Journal of medical toxicology. 8, (2012) 267-270, https://doi.org/10.1007/s13181-012-0229-z.

[41] P.S. CHu, S.C. Kwok, K.M. Lam, T.Y Chu, S.W. Chan, C.W. Homem, W.K. Ma, K.L. Chui, M.K. Yao, Y.C. Chan, M.L. Tse, F.L. Lau. Street ketamine'-associated bladder dysfunction: a report of ten cases. Hong Kong Medical Journal, 13, (2007) 311.

[42] Y. Kalkan, Y. Tomak, D. Altuner, L. Tumkaya, H. Bostan, A. Yilmaz, D. Unal, A. Kara, A. Turan. Hepatic effects of ketamine administration for 2 weeks in rats. Human & experimental toxicology, 33 (2014) 32-40, https://doi.org/10.1177/0 960327112472990 .

[43] M. Jang, J. Shen, J. Geng, H. Wang, S. Chuang, Y. Lee, C. Lee, Y. Lee, W. Wu, Y. Ju. Ketamine induced renal fibrosis in a ketamine addition rat model. Urological Science, 28 (2017) 123-127, https://doi.org/10.1016/j.urols.2016.12.004.

[44] V. R. Punithavathi, P.S.M. Prince, R. Kumar, J. Selvakumari. Antihyperglycaemic, antilipid peroxidative and antioxidant effects of gallic acid on streptozotocin induced diabetic Wistar rats. European Journal of Pharmacology. 650 (2011) 465–471, DOI:10.1016/j.ejphar.2010.08.059.

[45] F. Ng, M. Berk, O. Dean, A.I. Bush. Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. International Journal of Neuropsychophar- macology, 11 (2008) 851-876. DOI:10.1017/S146114570700 8401.

[46] J. Bouayed, H. Rammal, R. Soulimani. Oxidative stress and anxiety: relationship and cellular pathways. Oxidative medicine and cellular longevity, 2, (2009) 63-67.

[47] S. Maeng, C.A. Zarate. The role of glutamate in mood disorders: results from the ketamine in major depression study and the presumed cellular mechanism underlying its antidepressant effects. Current psychiatry reports, 9 (2007) 467-474.

[48] H.W. Dong, L.W. Swanson, L. Chen, M.S. Fanselow, A.W. Toga. Genomic–anatomic evidence for distinct functional domains in hippocampal field CA1. Proceedings of the National Academy of Sciences, 106, (2009) 11794-11799. DOI: 10.1073/pnas.0812608106.

[49] A.V. Tzingounis, J.I. Wadiche. Glutamate transporters: confining runaway excitation by shaping synaptic transmission. Nature Reviews Neuroscience, 8, (2007) 935. DOI 10.1038/nrn2274.

[50] T.M. Du Bois, X.F. Huang. Early brain development disruption from NMDA receptor hypofunction: relevance to schizophrenia. Brain research reviews. 53 (2007) 260-270. DOI: 10.1016/j.brainresrev.2006.09.001.

[51] F. Khakpai, M. Nasehi, A. Haeri-Rohani, A. EidI, M.R. Zarrindast. Scopolamine induced memory impairment; possible involvement of NMDA receptor mechanisms of dorsal hippocampus and/or septum. Behavioural brain research, 231, (2012) 1-10. DOI: 10.1016/j.bbr.2012.02.049.

[52] O.J. Onaolapo, O.Q. Ademakinwa, T.O. Olalekan, A.Y. Onaolapo. Ketamine-induced behavioural and brain oxidative changes in mice: an assessment of possible beneficial effects of zinc as mono- or adjunct therapy. Psychopharmacology. (2017). DOI: 10.1007/s00213-017-4666-x.

[53] G.S. Vasconcelos, N.C. Ximenes, C.N. De Sousa, T.Q. Oliveira. L.L. Lima, D.F. Lucena, C.S. Gama, D. Macêdo, S.M. Vasconcelos. Alpha-lipoic acid alone and combined with clozapine reverses schizophrenia-like symptoms induced by ketamine in mice: Participation of antioxidant, nitrergic and neurotrophic mechanisms. Schizophrenia Research. 165 (2015) 163-70. DOI:10.1016/j.schres.2015.04.017.

[54] A.T. Da Silva, C.F.A.J. Maia, A.S. Monte, I.Q.A. Góis, R.C. Cordeiro, S.M.M. De Jesus, L.R. Freitas, L.D. Freitas, M. Maes, D. Macêdo. Reversal of schizophrenia-like symptoms and immune alterations in mice by immunomodulatory drugs. Journal of psychiatric research, v. 84, p. 49-58, 2017.DOI10.1016/j.jpsychires .2016.09.017.

[55] P.I. Schimites, H. J. Segat, L.G. Teixeira, L.R. Martins, L.T. Mangini, P.S. Baccin, H.Z. Rosa, L.H. Milanesi, M.E. Burger, A.V. Soares. Gallic acid prevents ketamine-induced oxidative damages in brain regions and liver of rats. Neuroscience Letters, 714, (2020) 134560, 2020. DOI 10. 1016/j.neulet.2019.134 560.

[56] S. Cartágenes, L.M.P. Fernandes, T.C.V.S. Carvalheiro, T. M. De Sousa, A.R.Q. Gomes, M.C. Monteiro, R.D. Prediger. “Special K” Drug on Adolescent Rats: Oxidative Damage and Neurobehavioral Impairments. Oxidative medicine and cellular longevity (2019). DOI: 10.1155/2019/5452727.

[57] K. Isuzugawa, M. Inoue, Y. Ogihara. Catalase contents in cells determine sensitivity to the apoptosis inducer gallic acid. Biological and Pharmaceutical Bulletin, 24 (2001) 1022-1026.

[58] Y. Chandrasekhar, G.P. Kumar, E.M. Ramya, K.R. Anilakumar. Gallic acid protects 6-OHDA induced neurotoxicity by attenuating oxidative stress in human dopaminergic cell line. Neurochemical research. 43 (2018) 1150-1160.

[59] I. Izquierdo, M. Cammarota, W.C.D. Silva, L.R. Bevilaqua, J.I. Rossato, J.S. Bonini, J.H. Medina. The evidence for hippocampal long-term potentiation as a basis of memory for simple tasks. Anais da Academia Brasileira de Ciências. 80 (2008) 115-127. DOI:10.1590/S0001-37652008000100007.

[60] T.T. Duan, et al. Acute ketamine induces hippocampal synaptic depression and spatial memory impairment through dopamine D1/D5 receptors. Psychopharmacology. 228, (2013) 451-461. DOI: 10.1007/s00213-013-3048-2.

[61] B. Lu, P.T. Pang, N.H. Woo. H. Newton. The yin and yang of neurotrophin action. Nature Reviews Neuroscience, 6 (2005) 603. DOI: doi:10.1038/nrn1726.

[62] S. Kohtala, W. Theilmann, M. Rosenholm, H.K. Müller, P. Kiuru, G. Wegener, J. YLI-Kauhaluoma, T. Rantamäki. Ketamine-induced regulation of TrkB-GSK3β signaling is accompanied by slow EEG oscillations and sedation but is independent of hydroxynorketamine metabolites. Neuropharmacology. 107684 (2019) DOI: 10.1016/j.neuropharm.2019.107684.

[63] L. Nguyen, B.P. Lucke-Wold, A.F. Logsdon, A.L. Scandinaro, J.D. Huber, R.R. Matsumoto. Behavioral and biochemical effects of ketamine and dextromethorphan relative to its antidepressant-like effects in Swiss Webster mice. Neuroreport. 27 (2016) 1004. DOI 10.1097/WNR.0000000000000646.

[64] M.K. Harte, M. Cahir, G.P. Reynolds, J.E. Gartlon, D.N. Jones. Sub-chronic phencyclidine administration increases brain-derived neurotrophic factor in the RAT hippocampus. Schizophrenia research, 94 (2007) 371-372. DOI:10.1016/j. schres.2007.04.032.

[65] S. Maya, T. Prakash, D. Goli. Evaluation of neuroprotective effects of wedelolactone and gallic acid on aluminium-induced neurodegeneration: Relevance to sporadic amyotrophic lateral sclerosis. European journal of pharmacology. 835 (2018) 41-51. DOI:10.1016/j.ejphar.2018.07.058.

[66] X. Ke, Y. Ding, K. Xu, H. He, M. Zhang, D. Wang, X. Deng, X. Zhang, C. Zhou, Y. Liu, Y. Ning, N. Fan. Serum brain-derived neurotrophic factor and nerve growth factor decreased in chronic ketamine abusers. Drug and alcohol dependence. 142 (2014) 290-294. DOI:10.1016/j.drugalcdep.2014.06.043.

[67] T. Zhao, Y. Li, W. Wei, S. Savage, L. Zhou, D. Ma. Ketamine administered to pregnant rats in the second trimester causes long-lasting behavioral disorders in offspring. Neurobiology of disease. 68 (2014) 145-155, 2014. DOI: 10.1016/j.nbd.2014.02.009.

[68] D. Zuo, L. Lin, Y. Liu, C. Wang, J. Xu, F. Sun, L. Li, Z. Li, Y. Wu. Baicalin attenuates ketamine-induced neurotoxicity in the developing rats: Involvement of PI3K/Akt and CREB/BDNF/Bcl-2 pathways. Neurotoxicity research. 30, (2016) 159-172. DOI 10.1007/s12640-016-9611-y.

[69] H.H. Hansen, T. Briem, M. Dzietko, M. Sifringer, A. Voss, W. Rzeski, B. F. Thor, R. Heumann, A. Stepulak, P. Bittigau, C. Ikonomidou. Mechanisms leading to disseminated apoptosis following NMDA receptor blockade in the developing rat brain. Neurobiology of disease. 16, (2004) 440-453. DOI: 10.1016/j.nbd.2004.03.013.

[70] J.X. Zhu, J.L. Shan, W.Q. Hu, J.X. Zeng, J.C. Shu. Gallic acid activates hippocampal BDNF-Akt-mTOR signaling in chronic mild stress. Metabolic brain disease. 34 (2019) 93-101. DOI 10.1007/s11011-018-0328-x.

[71] G. Patki, N. Solanki, F. Atrooz, F. Allam, S. Salim. Depression, anxiety-like behavior and memory impairment are associated with increased oxidative stress and inflammation in a rat model of social stress. Brain research. 1539 (2013)73-86. DOI: 10.1016/j.brainres.2013.09.033.

[72] M .Scheller, J. Bufler, I. Hertle, H.J. Schneck, C. Franke, E. Kochs. Ketamine blocks currents through mammalian nicotinic acetylcholine receptor channels by interaction with both the open and the closed state. Anesthesia & Analgesia, 83, (1996) 830-836. DOI:10.1097/00000539-199610000-00031.

[73] G. Keilhoff, A. Becker, G. Grecksch, G. Wolf, H.G. Bernstein. Repeated application of ketamine to rats induces changes in the hippocampal expression of parvalbumin, neuronal nitric oxide synthase and cFOS similar to those found in human schizophrenia. Neuroscience. 126 (2004) 591-598. DOI 10.1016/j.neuroscience.2004.03.039.

[74] G. Corona, D. Vauzour, J. Hercelin, C.M. Williams, J.P. Spencer. Phenolic acid intake, delivered via moderate champagne wine consumption, improves spatial working memory via the modulation of hippocampal and cortical protein expression/activation. Antioxidants & redox signaling. 19, (2013) 1676-1689. DOI: 10.1089/ars.2012.5142.

[75] R. Deshmukh, V. Sharma, N. S. Meha, N. Sharma, K.L. Bedi . Amelioration of intracerebroventricular streptozotocin induced cognitive dysfunction and oxidative stress by vinpocetine--a PDE1 inhibitor. European journal of pharmacology. 620 (2009) 49-56. DOI:10.1016/j.ejphar.2009.08.027.

[76] P.D. Hansi, P.S. Mainzen. Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. Chemico-biological interactions. 179 (2009) 118-124. DOI :10.1016/j.cbi.2008.12.012.

[77] K. Polewski, S. Kniat, D. Slawinska. Gallic acid, a natural antioxidant, in aqueous and micellar environment: spectroscopic studies. Current Topics in Biophysics. 26 (2002) 217-227.

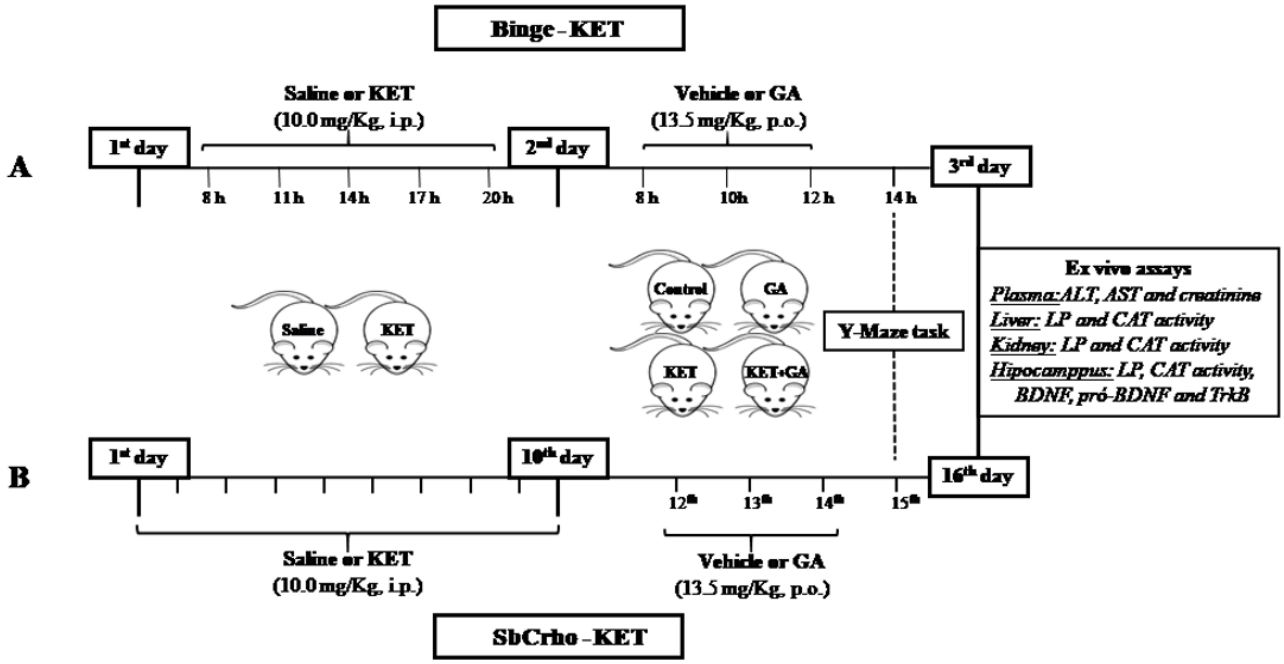


Figure 1. Experimental design: A) Binge-like protocol: animals were randomly divided in two groups (control or KET n = 12) and received five repeated doses of the drug (10 mg / kg, i.p.) or saline (0.9% NaCl, i.p.) simulating a recreational exposure (8h, 11h a.m., 14h, 17h, 20h p.m.). On the following day, half of each group was treated with three doses of GA (13.5 mg / kg, p.o.) or vehicle, corresponding to the following groups: Control; GA; KET; KET + AG (n = 6, per group). Two hours after the last administration of treatment, working memory and locomotion behavior were evaluated. On the third day, all animals were euthanized for additional biochemical and molecular analyses. B) Subchronic protocol: rats were randomly divided in two groups (n = 10-11) saline (0.9% NaCl, i.p.) or ketamine (10 mg / kg, i.p.), the animals received Saline or KET administration for 10 days in their respective groups. 48 hours after the animals were divided in the same experimental groups of the previous protocol and treated with GA (13,5 mg / kg, p.o.) or vehicle for three days (1dose/day). The next day, working memory and locomotion behavior were evaluated. 24 hours later, animals were euthanized for biochemical and molecular analysis. Abbreviations: GA: gallic acid; KET: ketamine; Binge-KET: ketamine binge; SbCrho-KET: subchronic ketamine; ALT: alanine transaminase; AST: aspartate transaminase; LP: lipid peroxidation;CAT:catalase.

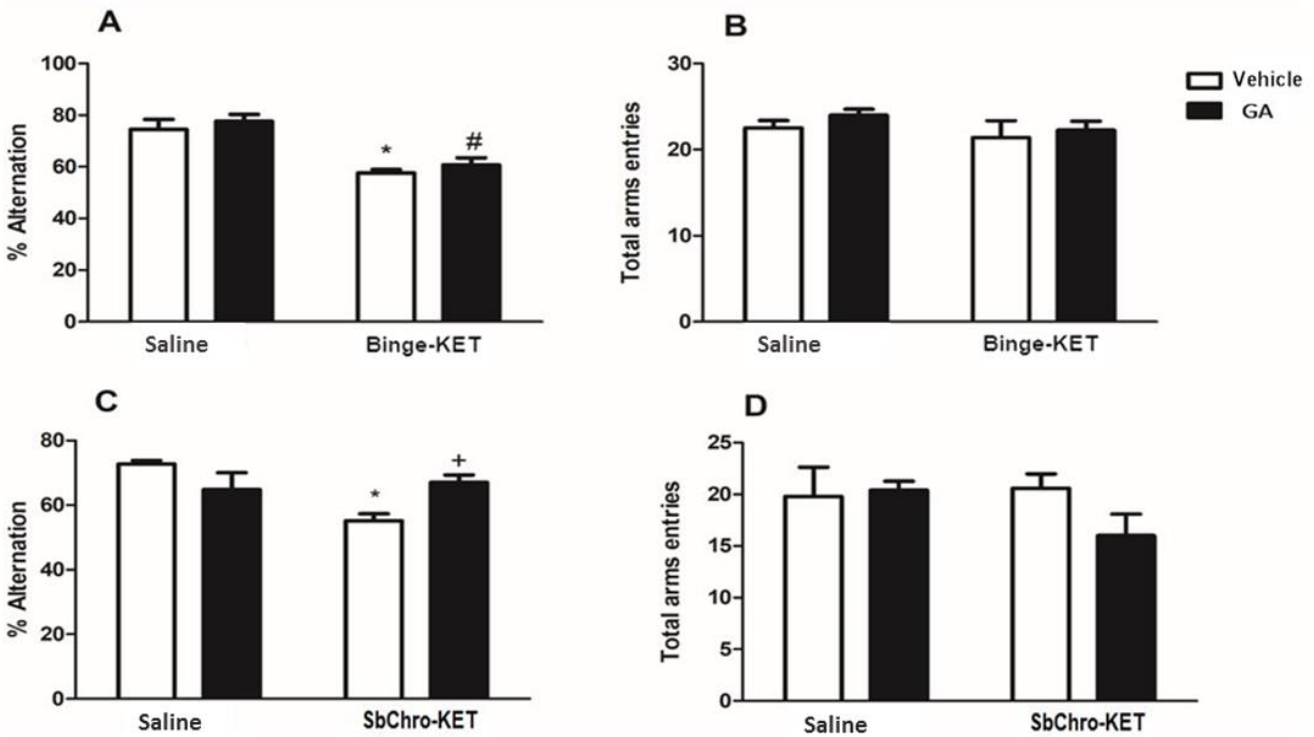


Figure 2. Influence of KET on working memory and locomotion measured in the Y-maze task by alternation rate and total arms entries. Rats were exposed to binge-like to KET and subsequently were treated with three doses of GA (2A, 2B), or the animals were exposed to SbChro-KET protocol and treated with three doses of GA (2C, 2D). Data are expressed as mean + S.E.M. * when compared to the control group values, + when compared to ketamine group and # when compared to GA group, considering $P < 0.05$. Abbreviations: GA: gallic acid; Binge-KET: ketamine binge; SbCrho-KET: subchronic ketamine.

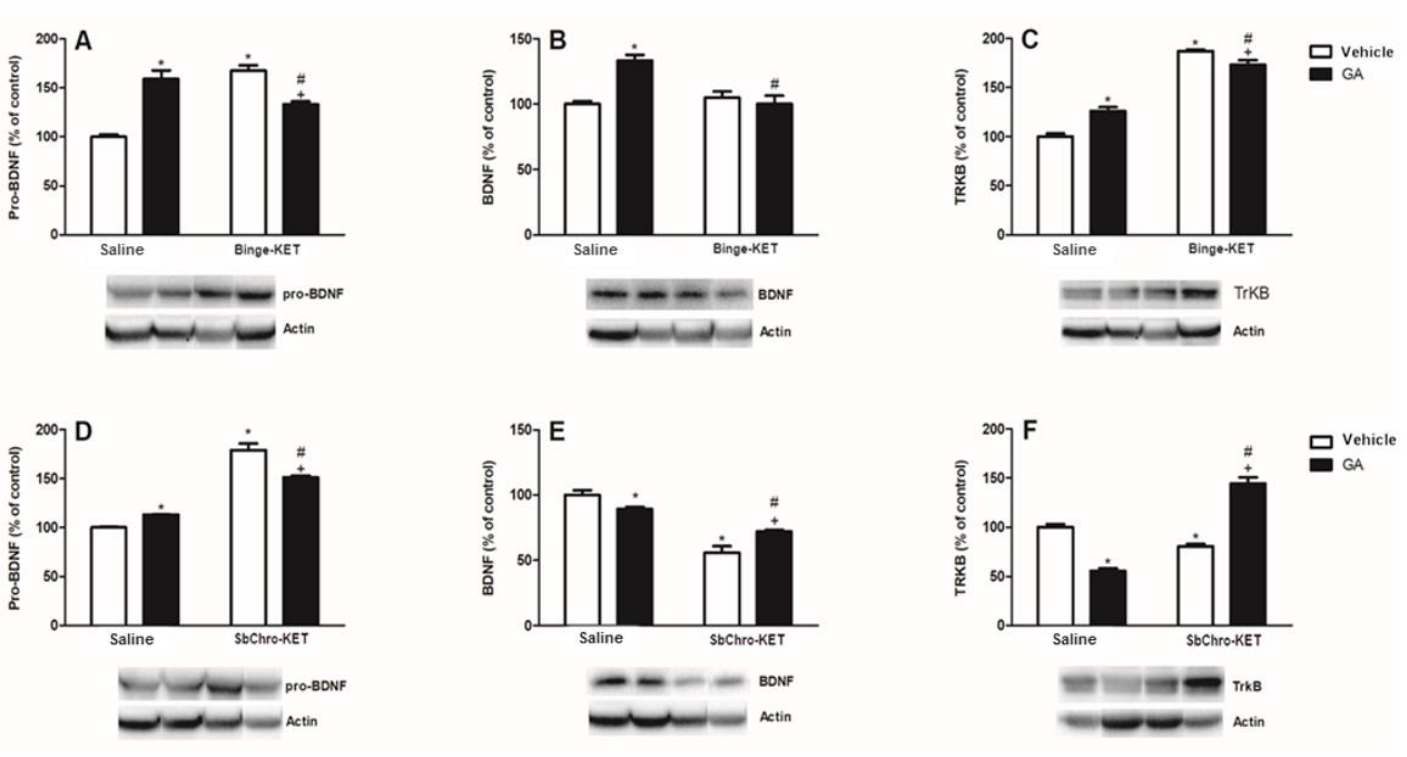


Figure 3. pro-BDNF, BDNFand TrkB immunoreactivity in hippocampus of rats exposed to binge-like to KET and subsequently were treated with three doses of GA (3A, 3B, 3C), or the animals were exposed to SbChro-KET protocol and treated with three doses of GA (3D, 3E, 3F). Each band in the sequence corresponds to one bar in the figure. Data are expressed as mean ± S.E.M. * when compared to the control group values, + when compared to ketamine group and # when compared to GA group, considering P <0.05. Abbreviations: GA: gallic acid; Binge-KET: ketamine binge; SbCrho-KET: subchronic ketamine.

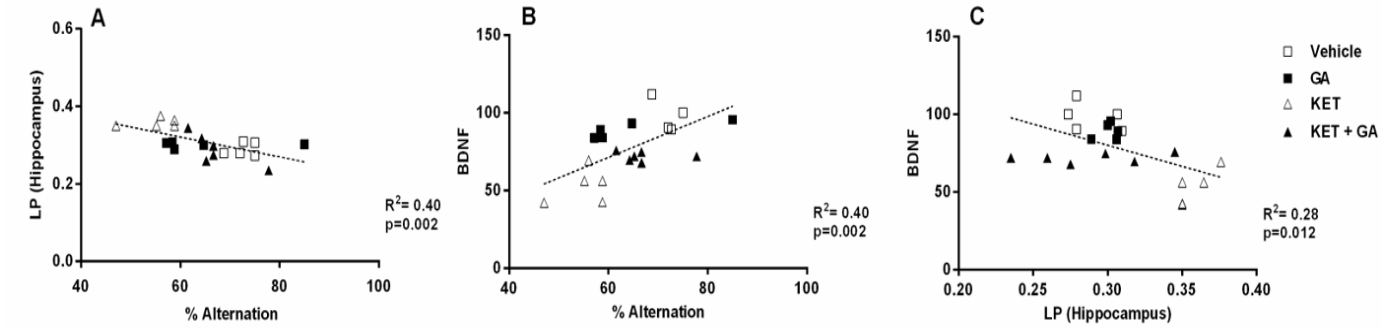


Figure 4. Correlations between the percentage of alternations in the Y-maze task and LP level in hippocampus (4A), the percentage of alternations in the Y-maze task and BDNF immunoreactivity (4B) and the LP level in hippocampus and BDNF immunoreactivity (4C) in animals which were exposed to SbChro-KET protocol and treated with three doses of GA. Abbreviations: GA: gallic acid; KET: ketamine; LP: lipid peroxidation.

Table 1. Biochemical parameters of creatinine and aminotransferases (ALT, AST) in plasma of animals exposed to KET-binge or subchronic-KET protocol. Subsenquently, the animal were treated with GA.

Groups	Creatinine (mg/dL)	ALT (U/L)	AST (U/L)
Binge-KET			
CONTROL	0.375 ± 0.01	38.40 ± 2.00	69.00 ± 4.60
GA	0.348 ± 0.01 *	40.40 ± 1.20	84.20 ± 3.65 *
KET	0.443 ± 0.00 *	37.40 ± 2.20	57.16 ± 4.10
KET+GA	0.376 ± 0.01 $^{\#+}$	46.20 ± 2.80 $^{+}$	74.48 ± 5.10 $^{+}$
SbChro-KET			
CONTROL	0.354 ± 0.00	38.60 ± 1.80	74.80 ± 8.90
GA	0.362 ± 0.02	45.60 ± 0.90	75.20 ± 2.30
KET	0.350 ± 0.02	64.40 ± 3.60*	119.4 ± 10.30*
KET+GA	0.386 ± 0.02	49.63 ± 3.20^{+}	96.50 ± 2.10 $^{\#+}$

Data are expressed as mean + S.E.M. in the table. * when compared to the control group values, $^{+}$ when compared to ketamine group and $^{\#}$ when compared to GA group, considering $p<0.05$. Abbreviations: GA: gallic acid; KET: ketamine; Binge-KET: ketamine binge; SbCrho-KET: subchronic ketamine; ALT: alanine transaminase; AST: aspartate transaminase.

Table 2. Oxidative stress parameters in the liver, kidney and hipocamppocampus of rats exposed to Binge-KET or SbChro-KET protocol. Subsenquently, the animal were treated with GA.

Groups		Liver	Kidney	Hippocampus
			Binge-KET	
CONTROL	**LP** (nmol MDA/g tissue)	36.11 ± 1.00	24.02 ± 0.40	40.97 ± 1.90
GA		25.30 ± 2.10*	21.66 ± 0.55	43.43 ± 1.10
KET		39.83 ± 2.90	29.24 ±1.80*	46.54 ± 0.80*
KET+GA		26.67 ± 1.90^{+}	22.51 ± 1.00^{+}	47.08 ± 1.30
			SbChro-KET	
CONTROL		100.00 ± 9.45	100.00 ± 6.80	28.90 ± 1.00
GA		104.49 ± 3.50	103.12 ± 3.20	30.00 ± 0.30
KET		129.12 ± 6.50*	130.73 ± 5.20*	35.80 ± 0.50*
KET+GA		102.50 ± 2.00^{+}	106.20 ± 4.10^{+}	28.80 ± 2.00^{+}
			Binge-KET	
CONTROL	**CAT activity** (mmol H_2O_2/min/g tissue)	1561.30 ± 112.60	1739.1 ± 71.30	319.37 ± 19.50
GA		877.71 ± 80.50*	1825.0 ± 81.00	177.48 ± 14.35*
KET		1846.0 ± 79.50*	1137.5 ± 60.30*	548.88 ± 47.60*
KET+GA		1517.6 ± 130.40$^{\#+}$	1229.1 ± 49.80$^{\#*}$	426.43 ± 34.00$^{\#+}$
			SbChro-KET	
CONTROL		1211.30 ± 293.40	955.44 ± 178.90	276.60 ± 26.50
GA		1262.02 ± 96.95	1155.98 ± 78.30	272.28 ± 98.70
KET		2009.76 ± 172.30*	2142.54 ± 433.40*	329.04 ± 73.95
KET+GA		1136.51 ± 123.25^{+}	846.14 ± 222.10^{+}	278.52 ± 73.95

Data are expressed as mean + S.E.M. * when compared to the control group values, $^{+}$ when compared to KET group and $^{\#}$ when compared to GA group, considering $p<0.05$. Abbreviations: GA: gallic acid; KET: ketamine;Binge-KET: ketamine binge; SbCrho-KET: subchronic ketamine; LP: lipid peroxidation levels; CAT: catalase activity.

6. CONCLUSÃO

Os resultados obtidos a partir do presente estudo demonstram a influência benéfica da administração de AG sobre a exposição aguda tipo *binge* e subcrônica de KET, permitindo-nos propor que:

- Os danos oxidativos induzido pela KET foram evidenciados em órgãos vitais como o rim, fígado e hipocampo em ambos protocolos agudo e subcrônico aqui desenvolvidos, entretanto tais danos foram mais claramente revertidos pelo AG quando a exposição à KET foi subcrônica;
- Análises moleculares realizadas na região do hipocampo revelaram que a KET aumentou a imunorreatividade do pró-BDNF e TrkB no protocolo de exposição aguda à droga, no entanto, tal tratamento foi capaz de reverter apenas parte destas alterações. Por outro lado, após a exposição subcrônica à KET, a imunoreatividade aumentada do pró-BDNF e diminuída do BDNF e TrkB foram observadas no hipocampo dos animais, no entanto todos esses marcadores foram revertidos na sua totalidade pelo tratamento com AG;
- A KET prejudicou a memória de trabalho nos dois protocolos aqui desenvolvidos, entretanto a administração de AG foi capaz de reverter tal dano apenas no protocolo subcrônico de exposição à KET.

Neste contexto, nossos resultados mostram que o AG reverte os danos oxidativos, moleculares e comportamentais induzidos pelo uso agudo abusivo e subcrônico de KET, permitindo-nos propor que este composto antioxidante natural possa, após estudos clínicos futuros, ser uma alternativa terapêutica útil na minimização de danos associados ao uso ilícito de KET.

REFERÊNCIAS

ANDRADE, V. M.; DOS SANTOS, F. H.; BUENO, O. F. **Neuropsicologia Hoje**., v. 27, n. 1, 2005. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-4 44 62005000100019> Acesso em: 12 de maio de 2019. DOI 10.1590/S1516-44462 0050001 00019.

ANNETTA, M. G.; IEMMA, D.; GARISTO, C.; TAFANI, C.; & PROIETTI, R. Ketamine: New indications for an old drug. **Current Drug Dargets**, v. 6, n.7, p. 789-794, 2005. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/16305457> Acesso em: 12 de maio de 2019. DOI 10.2174/138945005774574533.

BAJPAI, B.; PATIL, S. A new approach to microbial production of gallic acid. **Brazilian Journal of Microbiology**, v. 39, p. 708-711, 2008. Disponível em: <http:// www.scielo.b r/scielo.php?script=sci_arttext&pid=S1517-83822008000400021> Acesso em: 12 de maio de 2019. DOI 10.1590/S1517-83822008000400021.

BAKER, S. C. *et al*.Ketamine-induced apoptosis in normal human urothelial cells. **The American Journal of Pathology**. v. 186, n. 5, 2016.Disponível em: <https:/ /www. ncbi.nlm. nih. gov /pubmed/27001627>.Acesso em 23 de set. de 2018. DOI 10.1016/j.ajpath.2015.12.014.

BANNERMAN, D. M. *et al*. Distinct components of spatial learning reveated by prior training and NMDA receptor blockade. **Nature**. v. 378, p. 182-6, 1995. Disponível em:<https ://www.nature.com/articles/378182a0>. Acesso em: 21 de set. de 2018. DOI10.1038/37818 2a0.

BARRIOS, L. M. L.; PÉREZ, S. F. G. Uso de La ketamina epidural. Actualidad. **Gaceta Méd ica Espirituana**, v. 9, n. 1, p. 1, 2007. Disponível em: < http://www.revgmespirituana.sld.cu/i ndex.php/gme/article/view/696/535>. Acesso em 21 de janeiro de 2019.

BASCUÑANA, J. J. *et al*. Drogas dediseño: unnuevopaciente crítico. Manejo delasintoxicaci ciones por drogas consumidas enmacrofiestas. **Salud Rural**.v. 20, n. 3, p. 1-9, 2003.Disponível em: <http://www.scielo.br/scielo.php?script=sci_nlinks&ref=000066&pid=S0101-608320 0500010000200007&lng=es>. Acesso em: 23 de maio de 2018.

BRASIL. Portaria nº 344, de 12 de maio de 1998**.** Regulamento Técnico sobre substâncias e medicamentos sujeitos a controle especial. **Diário Oficial da União**, Brasília, DF, 19 de maio de 1998. Disponível em*:* <http: //www.anvisa .gov.br/scriptswe b/anvisalegis /Visualiza Documento.asp?ID=939&Versao=2> Acesso em 2 de jun. de 2019.

CARTÁGENES, S. C. *et al.*"Special K" Drug on Adolescent Rats: Oxidative Damage and Neurobehavioral Impairments. **Oxidative Medicine and Cellular Longevity**, v. 2019, 2019. Disponível em: <https://www.hindawi.com/journals/omcl/2019/5452727/abs/>.Acessoem: 21 de jun. de 2019. DOI 10.1155/2019/5452727.

CHAO, M. V.; RAJAGOPAL, R.; LEE, F. S. Neurotrophin signalling in health and disease. **Clinical science**, v. 110, n. 2, p. 167-173, 2006. Disponível em:< https ://www .ncbi. nlm.nih.gov/pubmed/16411893> Acesso em: 12 de maio de 2018. DOI 10.1042/CS20050163.

CHHILLAR, R.; DHINGRA, D. Antidepressant-like activity of gallic acid in mice subjected to unpredictable chronic mild stress.**Fundamental & Clinical Pharmacology**. v. 27, n. 4, p. 409-18, 2012. Disponível em <https://www.ncbi.nlm.nih.gov/pubmed/22458864>. Acesso em: 14 de mar.de 2018. DOI 10.1111/j.1472-8206.2012.01040.x.

CHU, J. *et al*. Adeno-associated virus-mediated brain delivery of 5-lipoxygenase modulates the AD-like phenotype of APP mice. **Molecular Neurodegeneration**, v.7, p.57-67, 2012. Disponível em:<https://www.ncbi.nlm.nih.gov/pubmed/22222029>. Acesso em: 8 de jan. de 2019. DOI 10.1186/1750-1326-7-1.

CRAVEN, R. Ketamine. **Anaesthesia**. v. 62, p. 48-53, 2007. Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/17937714>. Acesso em: 21 de maio de 2018. DOI 10.1111/j.1365-2044.2007.05298.x.

CRAWFORD, C. A. *et al*. Effects of monoamine depletion on the ketamine-induced locomotor activity of preweanling, adolescent, and adult rats: sex and age differences **Behavioural Brain Research**, p. 112267, 2019. Disponível em: < https://www.x-mol. com/paper/5883310> Acesso em 11 de jun. de 2019. DOI: 10.1016/j.bbr.2019.112267.

DAGLIA, M. *et al*. Polyphenols: well beyond the antioxidant capacity: gallic acid and related compounds as neuroprotective agents: you are what you eat. **Current Pharmaceutical Biotechnology.** v. 15, p. 362–372, 2014. Disponível em: <https:/ /www.ncbi.nlm.nih.gov/ pubmed/24938889>. Acesso em: 2 de maio de 2018.

DHINGRA, D.; CHHILLAR, R.; GUPTA, A.Antianxiety-like activityofgallicacidinunstres-sedand stressed mice: possible involvement of nitriergic system. **Neurochemical Research**, v. 37, n. 3, p. 487-494, 2012. Disponível em:<https://link.springer.com/article/10.1007/s11064-011-0635-7>. Acesso em: 12 de out. de 2018. DOI 10.1007/s11064-011-0635-7.

DHINGRA, M. S. *et al*. Effect of trimethylgallic acid esters against chronic stress-induced anxiety-like behavior and oxidative stress in mice. **Pharmacological Reports**, v. 66, n. 4, p. 606-612, 2014a. Disponível em: <https://www.sciencedirect.com/science/article/pii/S1734114014001492>Acesso em: 1 de maio de 2018. DOI 10.1016/j.pharep.2014.01.004.

DHINGRA, M. S. *et al*. Design, synthesis, physicochemical, and pharmacological evaluation of gallicacid esters as non-ulcerogenic andgastroprotectiveanti-inflammatory agents. **Medici-Nal Chemistry Research**, v. 23, n. 11, p. 4771-4788, 2014b. Disponível em: <https:// link. springer.com/article/10.1007/s00044-014-1041-x>. Acesso em: 2 de mar. de 2018. DOI10.1007/s00044-014-1041-x.

DILLON, P.; COPELAND, J.; JANSEN, K. Patterns of use and harms associated with non-medical ketamine use. **Drug and Alcohol Dependence**. v. 69, p. 23–8, 2003. Disponível em:<https://www.ncbi.nlmnih.gov/pubmed/12536063>.Acesso em: 15 de abr. de 2018.DOI 10.1016/s0376-8716(02)00243-0.

DOMINO, E. F. Taming the ketamine tiger. 1965. **Anesthesiology**. v. 113, p. 678–684, 2010. Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/20693870>. Acesso em 14 de jun. de 2018. doi:10.1097/ALN.0b013e3181ed09a2.

DOTSON, J. W.; ACKERMAN, D. L.; WEST, L. J. Ketamine abuse. Journal of Drug Issues. v. 25, p. 751-7, 1995. Disponível em:<https://journals.sagepub.com/doi/abs/10.1177/00220 4269502500407> Acesso em: 13 de mar. de 2019. DOI 10.1177/002204269502500407.

DU, Y. *et al*. Levo-tetrahydropalmatineinhibits the acquisition of ketamine-induced conditio-ned place preference by regulating the expression of ERK and CREB phosphorylation in rats. **Behavioral Brain Research**.v.317, p. 367-373, 2017. Disponível em: <https ://www. science direct.com/science/article/pii/S0166432816307586?via%3Dihub>. Acesso em 2 ago.de 2019. DOI 10.1016/j.bbr.2016.10.001.

DUAN, T. T. *et al*. Acute ketamine induces hippocampal synaptic depression and spatial memory impairment through dopamine D1/D5 receptors. **Psychopharmacology**, v. 228, n. 3, p. 451-461, 2013. Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/23494232>. Acesso em: 2 de jun. de 2018. DOI 10.1007/s00213-013-3048-2.

DUVAL, N. G. F. Anestésicos venosos. in: MANICA, J. (eds.). **Anestesiologia princípios e técnicas**. 3. ed. Porto Alegre: Artmed, 2004, p.560-97. Disponível em:<https://www.docsity. com/pt/anestesiologia-principios-e-tecnicas-3-edicao-james-manica-1434p/4877294/> Acesso em: 23 de jul. de 2018.

EIDE, P. K. *et al*. Ketamine: relief from chronic pain through actions at the NMDA receptor. **Pain**. v. 72, p. 289-291, 1997. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed /9 272819> Acesso em: 14 de abr. de 2018. DOI10.1097/00006396-199708000-00042.

FANTONI, D. T.; CORTOPASSI, S. R. G.; BERNARDI, M. M. Anestésicos intravenosos e outros parenterais. In: **Farmacologia Aplicada à Medicina Veterinária**. São Paulo: Guanabara Koogan, cap. 11, p. 132-143, 2006. Disponível em: <https ://bdpi.usp. br/item/ 00 1272932> Acesso em 23 de jun. de 2018.

FARBOOD, Y. *et al*. The effects of gallic acid on pain and memory following transient global ischemia/reperfusion in Wistar rats. **Avicenna Journal of Phytomedicine**, v. 3, n. 4, p. 329, 2013. Disponível em: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4075730/> Acesso em: 23 de jun. de 2018.

FDA - Food and Drug Administration. FDA approves new nasal spray medication for treatment-resistant depression; available only at a certified doctor's office or clinic, 2019 **U.S. FDA**. Disponível em:<https://www.fda.gov/news-events/press-announcements/fda-approves-new-nasal-spray-medication-treatment-resistant-depression-available-only-certified> Acesso em: 12 de jul. de 2019.

FERNANDES, F. H. A.; SALGADO, H. R. N. Gallic acid: review of the methods of determination and quantification. **Critical Reviews in Analytical Chemistry**, v. 46, n. 3, p. 257-265, 2016. Disponível em:<https://www.tandfonline.com/doi/abs/10.1080/10408347. 2015.1095064> Acesso em 2 de abr. de 2018. DOI 10.1080/10408347.2015.1095064.

FISHER, K.; CODERRE, T. J.; HAGEN, N. A. Targeting the N-methyl-D-Aspartate receptor for chronic pain management preclinical animal studies recent clinical experience and future research directions. **Journal of Pain and Symptom Manage**. v. 20, p. 358-73, 2000. Dispon-ível em:<https://www.ncbi.nlm.nih.gov/pubmed/11068158>Acessoem: 1 de set. de 2018. DOI 10.1016/S0885-3924(00)00213-X.

FREESE, T. E.; MIOTTO, K.; REBACK, C. J. The effects and consequences of selected club drugs. **Journal of Substance Abuse Treatment**. v. 23, p. 151-6, 2002. Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/12220613>Acesso em 27 de maio de 2018. DOI 10.1016/S0740-5472(02)00267-2.

GALES, A.; MAXWELL, S. Cetamina: Evidências Recentes e Usos Atuais. **Anaesthesia Tutorial of the Week**. Disponível em: < https://www.sbahq.org/wp-content/ uploads/ 2018/07/381-portugues.pdf > Acesso em 3 de jun. de 2019.

GARCIA, J. B. S. Cetamina uma nova leitura. **Prática Hospitalar**. v. 53, p. 214-6, 2007. Disponível em: <http://www.scielo.br/scielo.php?script=sci_nlinks&ref=000139&pid=S0034-70942012000500006000022&lng=pt> Acesso em: 24 de maio de 2018.

GHAZNAVI, H. *et al*. Ameliorative effects of gallicacid on gentamicin-induced nephron toxicity in rats. **Journal of Asian Natural Products Research**, v. 20, n. 12, p. 1182-1193, 2017. Disponível em:<https://www.tandfonline.com/doi/abs/10.1080/10286020.2017.1384819> Acesso em: 2 de maio de 2019. DOI 10.1080/10286020.2017.1384819.

GLABE, R. S. Acute toxic effects of club drugs. **Journal of Psychoactive Drugs**. v. 36, p. 303-13, 2004. Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/15559678> Acesso em: 12 de maio de 2018. DOI: 10.1080/02791072.2004.10400031.

GOODMAN, L. S.; GILMAN, A. G.; BRUNTON, L. L. **As bases farmacológicas da terapêutica**. 12 Ed, 2015, cap. 19.

GONDA, X. Basic pharmacology of NMDA receptors. **Current Pharmaceutical Design**. v.18, n. 5, p. 1558-1567, 2012. Disponível em:<https://www.ncbi.nlm.nih.gov/pubmed/22280436> Acesso em: 14 de maio de 2019. DOI 10.2174/138161212799958521.

HALLIWELL, B.; WHITEMAN, M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean?. **British Journal of Pharmacology**, v. 142, n. 2, p. 231-255, 2004. Disponível em: <https://www.ncbinlm.nih.gov/pubmed/15155533> Acesso em: 2 de out. de 2018. DOI 10.1038/sj.bjp.0705776.

HANSI, P. D.; STANELY, P. P. Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. **Chemico-BiologicalInteractions**., v. 179, p. 118-124, 2009. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/19146839. Acesso em: 13 de out.de 2018. DOI 10.1016/j.cbi.2008.12.012.

HEMELRIJCK, J. V.; WHITE, P. NonopioidIntravenousAnesthesia. In: Barash PG, Cullen BF, Stoelting RK (eds.). **Clinical Anesthesia**. 3th ed. Philadelphia: Lippincott-Raven. p.312-27, 1997. Disponívelem:<http://www.scielo.br/scielo.php?script=scinlinks&ref=000084&pid=S0034-70942005000400011000006&lng=en> Acesso em 2 de maio de 2018.

HUANG, L. *et al*. Ketamine potentiates hippocampal neurodegeneration and persistent learning and memory impairment through the PKCγ–ERK signaling pathway in the developing brain. **Brain research**, v. 1476, p. 164-171, 2012. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0006899312013005> Acesso em: 13 de mar. de 2018. DOI 10.1016/j.brainres.2012.07.059

JANG, M. *et al*. Ketamine induced renal fibrosis in a ketamine addition rat model. **Urological Science**, v. 28, n. 3, p. 123-127, 2017. Disponìvel em: <https://cyberleninka.org/article/n/739623.pdf > Acesso em: 12 de maio de 2019. DOI 10.1016/j.urols.2016.12.004.

JANSEN, K. L.; DARRACOT-CANKOVIC, R. The nonmedical use of ketamine, part two: A review of problem use and dependence. **Journal Psychoactive Drugs**. v. 33, p. 151–8, 2001. Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/11476262> Acesso em 21 de set. de 2018. DOI 10.1080/02791072.2001.10400480.

KALKAN, Y.; TOMAK, Y.; ALTUNER, D.; TUMKAYA1, L.; BOSTAN, H.; YILMAZ, A; UNAL, D.; KARA, A.; TURAN, A. Hepatic effects of ketamine administration for 2 weeks in rats. **Human and Experimental Toxicology,** v. 33, p. 32-40, 2014. Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/23386779> Acesso em 20 de maio de 2019. DOI 10.1177 /0960327112472990.

KE, X. *et al*. Serum brain-derived neurotrophic factor and nerve growth factor decreased in chronic ketamine abusers. **Drug and alcohol dependence**, v. 142, p. 290-294, 2014. Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/25064020> Acesso em: 13 de maio de 2018. DOI 10.1016/j.drugalcdep.2014.06.043

KIM, Y. Antimelanogenicand antioxidant properties of gallicacid. **Biological & Pharmaceutical Bulletin**. v. 30, p. 1052-1055, 2007.Disponível em:<https://www.ncbi.nlm.nih.gov/pubmed/17541153> Acesso em: 4 de mar. de 2019. DOI 10.1248/bpb.30.1052.

KOHRS, R.; DURIEUX, M. E. Ketamine: teaching an old drug new tricks. **Anesthesia & Analgesia**. V. 87, p. 1186-93, 1998. Disponível em: <https://www.ncbi.nlm.nih.gov/ pubmed/9806706> Acesso em: 12 de out. de 2018. DOI 10.1097/00000539-199811000-00039.

KROES, B. H. *et al.* Anti-inflammatory activity of gallic acid. **Planta medica**, v. 58, n. 06, p. 499-504, 1992. Disponível em: <https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-2006-961535> Acesso em: 2 de jun. de 2018. DOI 10.1055/s-2006-961535.

LI, Y. *et al*. Effects of ketamine on levels of inflammatory cytokines IL-6, IL-1β, and TNF-α in the hippocampus of mice following acute or chronic administration. **Frontiers in Pharmacology**, v. 8, p. 139, 2017. Disponível em< https ://www.ncbi.nlm. nih. gov/pmc /articles/PMC5357631/> Acesso em: 15 de jun. de 2019. DOI 10.3389/fphar.2017.00139.

LIMA, K. G. *et al*. Gallic acid reduces cell growth by induction of apoptosis and reduction of IL-8 in HepG2 cells.**Biomedicine & Pharmacotherapy**. v. 84, p. 1282–1290, 2016. Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/27810785> Acesso em: 2 de maio de 2018. DOI doi: 10.1016/j.biopha.2016.10.048.

LIU, Y. *et al*. Limb Remote Ischemic Preconditioning Reduces Repeated Ketamine Exposure-Induced Adverse Effects in the Developing Brain of Rats. **Journal of Molecular Neuroscience**, v. 68, n. 1, p. 58-65, 2019. Disponível em:<https://link.springer.com/article/10.1007/s12031-019-01282-3> Acesso em: 2 de jun. de 2019. DOI 10.1007/s12031-019-01282-3.

LU., Z. et al. Structure-activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives, **Neurochemistry International.** v. 48, p.263–274, 2006. DOI 10.1016/j.neuint.2005.10.010.

LUFT, A.; MENDES, F. F. S (+) Cetamina em baixas doses: atualização. **Revista Brasileira de Anestesiologia**. v. 55, p. 460- 9, 2005. Disponível em: <http ://www. scielo .br/ scielo .php ?script=sci_arttext&pid=S0034-70942005000400011> Acesso em: 2 de mar. de 2019. DOI 10.1590/S0034-70942005000400011.

MACHADO, A. B. M. **Neuroanatomia funcional**. 2ª ed. São Paulo. Atheneu. p. 229-235, 2006. Diponível em: <https://www.academia.edu/20437017/Livro-neuroanatomia-funcional-2ed-angelo-machado> Acesso em 18 de out. de 2018.

MANSOURI, M. T.*et al*. Neuroprotective effects of oral gallic acid against oxidative stress induced by 6-hydroxydopamine in rats. **Food Chemistry**, v. 138, n. 2-3, p. 1028-1033, 2013.Disponível em:<https: //www.sciencedirect .com/science /article/pii /S0308814612017 499> Acesso em: 3 de maio de 2018. DOI 10.1016/j.foodchem.2012.11.022.

MORGAN, C. J; CURRAN, H. V. INDEPENDENT SCIENTIFIC COMMITTEE ON DRUGS (ISCD). Ketamine use: a review. **Addiction**, v. 107, n. 1, p. 27-38, 2012. Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/21777321> Acesso em: 13 de maio de 2018. DOI 10.1111/j.1360-0443.2011.03576.x.

NATIONAL INSTITUTES OF HEALTH. **PubChem**. Compound Summary for CID 370, 2015. Disponível em: <https://pubchem.ncbi.nlm.nih.gov/compound/Gallic-acid> Acesso em: 14 de mar. de 2019.

OLIVEIRA, C. M. B. *et al*. Cetamina e Analgesia Preemptiva. **Revista Brasileira de Anestesiologia**. v. 54, p. 739-52, 2004. Disponível em: <http://www.scielo.br/pdf/rba/v54n5 /v54n5a16.pdf> Acesso em: 11 de maio de 2018. DOI 10.1590/S0034-70942004000500016.

ONAOLAPO, A. Y. *et al*. Subchronic ketamine alters behaviour, metabolic indices and brain morphology in adolescent rats: Involvement of oxidative stress, glutamate toxicity and caspase-3-mediated apoptosis. **Journal of Chemical Neuroanatomy**, v. 96, p. 22-33, 2019. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0891061818301443> Acesso em: 3 de maio de 2018. DOI 10.1016/j.jchemneu.2018.12.002.

ONAOLAPO, O.J., *et al*. Ketamine-induced behavioural and brain oxidative changes in mice: an assessment of possible beneficial effects of zinc as mono- or adjunct therapy. **Psychopharmacology.** 2017. Disponível em: <https: //www. ncbi. nlm.nih. gov/pubmed /28 612134>Acesso em: 14 de nove. de 2018. DOI 10.1007/s00213-017-4666-x.

ORANJE, B. *et al*. The effects of a sub-anaesthetic dose of ketamine on human selective attention. **Neuropsychopharmacology**. v. 22, p. 293-302, 2000. Disponível em: <https://www .ncbi.nlm.nih.gov/pubmed/10693157> Acesso em: 23 de maio de 2018. DOI 10.1016/S0893-133X(99)00118-9.

PAULE, M. G. *et al*. Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys. **Neurotoxicology and Teratology**. v. 33, p. 220-30, 2011.

Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/21241795> Acesso em: 12 de abr. de 2018. DOI 10.1016/j.ntt.2011.01.001.

POLEWSKI, K.; KNIAT, S.; SLAWINSKA, D. Gallic acid, a natural antioxidant, in aqueous and micellar environment: spectroscopic studies. **Current Topics in Biophysics**., v. 26, p. 217-227, 2002. Disponível em: <https://www.researchgate.net/profile/Krzysztof_Polewski /publication/284542596_Gallic_acid_a_natural_antioxidant_in_aqueous_and_micellar_environment_spectroscopic_studies/links/5b1e9b4745851587f2a079e9/Gallic-acid-a-natural-antioxidant-in-aqueous-and-micellar-environment-spectroscopic-studies.pdf> Acesso em: 21 de maio de 2018.

RAEDER, J. C.; STENSETH, L. B. Ketamine: a new look at an old drug. **Current Opinion in Anesthesiology**, v. 13, p. 463-8, 2000. Disponível em:<https://www.ncbi.nlm.nih.gov/pubmed/12768186> Acesso em: 2 de maio de 2018.

RANG, H. P.; DALE, M. M.; RITTER, J. M. **Farmacologia**. 6ª. ed. Rio de Janeiro: Elsevier, 2007, 920p. Disponível em: <http://www.scielo. br/scielo.php? script=scinlinks&ref =000186&pid=S1809-98232011000200008000019&lng=pt>Acesso em: 12 de mar. de 2019.

RASOOL, M. K. *et al*. Hepatoprotective and antioxidant effects of gallic acid in paracetamol-induced liver damage in mice. **Journal of Pharmacy and Pharmacology**. v. 62, p. 638–643, 2010. Disponível em:<https://www.ncbi.nlm.nih.gov/pubmed/20609067> Acesso em: 19 de maio de 2018. DOI 10.1211/jpp.62.05.0012.

RECKZIEGEL, P. *et al*. Antioxidant protection of gallic acid against toxicity induced by Pb in blood, liver and kidney of rats. **Toxicology Reports.** v. 3, p. 351-356, 2016. Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/28959556> Acesso em 14 de maio de 2018. DOI 10.1016/j.toxrep.2016.02.005.

RECKZIEGEL, P. *et al*. Locomotor damage and brain oxidative stress induced by lead exposure are attenuated by gallic acid treatment, **Toxicological. Letters**. v. 203, p. 74–81, 2011. Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/21402136> Acesso em: 13 de maio de 2018. DOI 10.1016/j.toxlet.2011.03.006.

RÉUS, G.Z., *et al*. Ketamine potentiates oxidative stress and influences behavior and inflamemation in response to lipolysaccharide (LPS) exposure in early life. **Neuroscience**, v. 353, p.17–25, 2017.Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/28433652> Acesso em: 9 de maio de 2018. DOI 10.1016/j.neuroscience.2017.04.016.

ROCHA, W. S. *et al*. Compostos fenólicos totais e taninos condensados em frutas nativas do cerrado. **Revista Brasileira de Fruticultura**, v. 33, n. 4, p. 1215-1221, 2011. Disponível em: < http://www.scielo.br/scielo.php?pid=S010029452011000400021&script=sci_abstract&tlng=pt> Acesso em: 14 de jun. de 2019. DOI 10.1590/S0100-29452011000400021.

SAFAEI, F., *et al*. Protective effects of gallic acid against methotrexate-induced toxicity in rats. **Acta Chirurgica Belgica**, v. 118, p. 152–160, 2018. Disponível em: <https: //www .ncbi.nlm.nih.gov/pubmed/29069994> Acesso em: 12 de maio de 2018. DOI 10 .1080/ 00015458. 2017.1394672.

SAMPAIO, T. B. *et al.* Long-Term neurobehavioral consequences of a single ketamine neonatal exposure in rats: effects on cellular viability and glutamate transport in frontal cortex and hippocampus.**Neurotoxicity Research**. v. 34, p. 649-659, 2018. Disponível em: <https:// www.ncbi.nlm.nih.gov/pubmed/29968149>Acesso em: 23 de mar. de2019. DOI10.1007/s12 640-018-9927-x.

SARKAKI, A. *et al*. Gallic acid improved behavior, brain electrophysiology, and inflammation in a rat model of traumatic brain injury. **Canadian Journal of Physiology and Pharmacology**. v. 93, p. 1-8, 2015. Disponível em:< https:/ /www.ncbi.nlm.nih.gov/pubmed /26222320>Acesso em: 21 de jun. De 2018. DOI 10.1139/cjpp-2014-0546.

SHIBUTA, S. *et al*. Only extra-high dose of ketamine affects l-glutamate-induced intracellular Ca2+ elevation and neurotoxicity. **Neuroscience Research**, v. 98, p. 9-16, 2015. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0168010215001327> Acesso em: 3 de jun. de 2018. DOI 10.1016/j.neures.2015.04.005.

SIEGEL, R. K. Phencyclidine and ketamine intoxication: a study of four populations of recreational users. **NIDA Research Monograph**. v. 21, p. 119-47, 1978. Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/101865> Acesso em: 14 de maio de 2018.

SILVA, F. C. C. *et al*. Ketamina, da anestesia ao uso abusivo: artigo de revisão**. Revista de Neurociências**.v.18, p.277-237, 2010. Disponível em: <http://www.revistaneurociencias.com .br/edicoes/2010/RN1802/349%20revisao.pdf> Acesso em: 15 de jun. de 2018.

SORIANO, S. G. *et al*. Ketamine activates cell cycle signaling and apoptosis in the neonatal rat brain. **Anesthesiology**. V. 112, p. 1155-63, 2010. Disponível em: <https: //www .ncbi. nlm .nih.gov/pubmed/20418696> Acesso em: 18 de maio de 2019. DOI 10.1 097 /ALN .0b 013 e 3181d3e0c2.

SUBRAMANIAN, V.*et al*. Topical application of Gallic acid suppresses the 7, 12-DMBA /Croton oil induced two-step skin carcinogenesis by modulating antioxidants and MMP-2/M MP-9 in Swiss albino mice. **Food and Chemical Toxicology**, v. 66, p. 44-55, 2014. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0278691514000313> Acesso em: 12 de jun. de 2018. DOI 10.1016/j.fct.2014.01.017.

TRUJILLO, K. A.; ZAMORA, J. J.; WARMOTH, K. P. Increased Response to Ketamine Following Treatment at Long Intervals: Implications for Intermittent Use. **Biological Psychiatry.** v. 63, p. 178-83, 2008. Disponível em:<https://www.sciencedirect.com/science/ article/pii/S0006322307001503?via%3Dihub> Acesso em: 12 de amio de 2019. DOI 10.1016/j.biopsych.2007.02.014.

UNITED NATIONS OFFICE ON DRUGS AND CRIME (UNODC). **World Drug Report.** (United Nationspublication, Sales No. E.14.XI.7, 2014. Disponível em: <https:/ /www. unodc. org/documents/lpo-brazil/ noticias/ 2014/ 06/ World Drug Report. 2014_web embargoed.pdf> Acesso em: 23 de maio de 2018.

UHLHAAS, P. J.*et al*. Perceptual organization in ketamine users: preliminary evidence of deficits on night of drug use but not 3 days later. **Journal of Psychopharmacology**.v. 21, p. 347–52, 2007. Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/17591661> Acesso em: 24 de jun. de 2018. DOI 10.1177/0269881107077739.

VASCONCELOS, M. M. S. *et al*. Cetamina: aspectos gerais e relação com a esquizofrenia**.** **Revista de Psiquiatria Clínica**, v. 32, n. 1, p. 10-16, 2005.Disponível em:<http://www.scielo .br/scielo.php?script=sci_arttext&pid=S0101-60832005000100002> Acesso em: 11 de maio de 2019. DOI 10.1590/S0101-60832005000100002.

VALADÃO, C. A. A. Anestésicos dissociativos. In: FANTONI, D. T.; CORTOPASSI, S. R. G. **Anestesia de Cães e Gatos**. 2ª ed. São Paulo: Roca, p. 237-245, 2010. Disponível:< http ://www.scielo.br/scielo.php?script=sci_nlinks&ref=000090&pid=S0102-09352007000300022 00020&lng=en> Acesso em: 21 de nov. 2018.

WANG, C. *et al*. Blockade of N-methyl-D-aspartate receptors by ketamine produces loss of postnatal day 3 monkey frontal cortical neurons in culture. **Toxicological Sciences**, v. 91, n. 1, p. 192-201, 2006. Disponível em: <https://academic.oup.com/toxsci/article/91/1/192/167 2704> Acesso em 14 de maio de 2018. DOI 10.1093/toxsci/kfj144.

WORLD DRUG REPORT(WDR). **United Nations publication**, Sales No. E.18.XI.9, 2018. Disponível em: <https ://www.unodc. org/wdr2018/prelaunch/ WDR18_ Booklet_1_ EXSUM .pdf> Acesso em: 15 de mar. de2019.

YADAV, M.*et al*. Protective effect of gallic acid in experimental model of ketamine-induced psychosis: possible behaviour, biochemical, neurochemical and cellular alterations. **Inflam mopharmacology**.v. 26, p. 413-424, 2017. Disponível em: <https: // www .ncbi. nlm .nih.gov/pubmed/28577133> Acesso em: 13 de mar. de 2019.DOI 10.1007/s10787-017-0366-8.

ZHAO, T. *et al*. Ketamine administered to pregnant rats in the second trimester causes long-lasting behavioral disorders in offspring. **Neurobiology of Disease**, v. 68, p. 145-155, 2014. Disponível em: <https ://www.sciencedirect. com/science/article/pii/S0969996114000515> Acesso em: 2 de maio de 2018. DOI 10.1016/j.nbd.2014.02.009.

ZOU, X. *et al*. Potential neurotoxicity of ketamine in the developing rat brain. **Toxicological sciences**, v. 108, n. 1, p. 149-158, 2009. Disponível em: <https: //academic. oup.com/ toxsci/ article/108/1/149/1674581> Acesso em: 13 de maio de 2018. DOI 10.1093/toxsci/kfn270.

ZUGNO, A. I. *et al.* Chronic exposure to cigarette smoke during gestation results in altered cholinesterase enzyme activity and behavioral deficits in adult rat offspring: potential relevance to schizophrenia**. Journal of Psychiatric Research.**v. 47, n. 6, p. 740-746, 2013. Disponível em: <https://www.ncbi.nlm.nih.gov/pubmed/23472836> Acesso em: 14 de jun. de 2018. DOI 10.1016/j.jpsychires.2013.02.001.

ZUO, D. *et al*. Baicalin attenuates ketamine-induced neurotoxicity in the developing rats: Involvement of PI3K/Akt and CREB/BDNF/Bcl-2 pathways. **Neurotoxicity Research**, v. 30, n. 2, p. 159-172, 2016. Disponível em: <https://link.springer.com/article/10.1007/s12640-016-9611-y> Acesso em: 4 de maio de 2018. DOI 10.1007/s12640-016-9611-y.

REFERÊNCIAS DE IMAGEM

Figura 1: https://paginateste123.wordpress.com/caracteristicas-estruturais/
Figura 2: http://principo.org/questo-01-uerj2016.html

ANEXO I – CERTIFICADO DE APROVAÇÃO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA UNIVERSIDADE FEDERAL DE SANTA MARIA

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "POSSÍVEL ATIVIDADE NEUROPROTETORA DO ÁCIDO GÁLICO E DO ISOTERÁPICO DE CETAMINA SOB PARÂMETROS COMPORTAMENTAIS E OXIDATIVOS INDUZIDOS POR CETAMINA EM RATOS", protocolada sob o CEUA nº 8629131218 (ID 002467), sob a responsabilidade de **Marilise Escobar Bürger** *e equipe; Marilise Escobar Bürger; Gerson Fernandes de Brum; Laura Hautrive Milansei; Vinícia Garzella Metz; Hecson Jesser Segat; Domenika Rubert Rossato; Jessica Leandra Oliveira da Rosa* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 07/05/2019.

We certify that the proposal "POSSIBLE NEUROPROTECTIVE ACTIVITY OF GALLIC ACID AND ISOTERAPIC OF KETAMINE ON THE BEHAVIORAL AND OXIDATIVE PARAMETERS KETAMINE-INDUCED IN RATS ", utilizing 84 Heterogenics rats (84 males), protocol number CEUA 8629131218 (ID 002467), under the responsibility of **Marilise Escobar Bürger** *and team; Marilise Escobar Bürger; Gerson Fernandes de Brum; Laura Hautrive Milansei; Vinícia Garzella Metz; Hecson Jesser Segat; Domenika Rubert Rossato; Jessica Leandra Oliveira da Rosa* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 05/07/2019.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 05/2019 a 12/2021 Área: Farmacologia

Origem:	Biotério Central UFSM						
Espécie:	Ratos heterogênicos	sexo:	Machos	idade:	21 a 45 dias	N:	84
Linhagem:	Wistar			Peso:	60 a 150 g		

Local do experimento: Todas as etapas serão realizadas no prédio 21. O animais serão mantidos no biotério e para os procedimentos experimentais e comportamentais serão transferidos para a sala 5220.

Santa Maria, 02 de dezembro de 2019

Profa. Dra. Patrícia Severo do Nascimento
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Avenida Roraima, 1000, Reitoria, 2º andar - CEP 97105-900 Santa Maria, RS - tel: 55 (55) 3220-9362
Horário de atendimento: das 8:30 às 12h e 14h às 17hs : e-mail: ceua.ufsm@gmail.com
CEUA Nº 8629131218