Acta Veterinaria Brasilica December 14 (2020) 259-264

Acta Veterinaria Brasilica

Journal homepage: https://periodicos.ufersa.edu.br/index.php/acta/index

Original Articles

Evaluation of the effects of acepromazine and xylazine on viability in an equine dermal cell line

Avaliação dos efeitos de acepromazina e xilazina na viabilidade de linhagem celular dérmica de equino

Jéssica Grace da Silveira[1,3], Bruno Egídio Cappelari[1,4], Ana Paula Muterle Varela[5], Thais Fumaco Teixeira[5], Giovana Dantas de Araujo [1,2*]

[1] Laboratório de Virologia. Instituto de Pesquisas Veterinárias Desidério Finamor. Departamento de Diagnóstico e Pesquisa Agropecuária. Secretaria da Agricultura, Pecuária e Desenvolvimento Rural do Rio Grande do Sul.
[2] Programa de Pós-graduação em Saúde Animal do Instituto de Pesquisas Veterinárias Desidério Finamor. Rio Grande do Sul, Brasil.
[3] Centro Universitário Ritter dos Reis. Porto Alegre, Rio Grande do Sul, Brasil.
[4] Universidade do Vale do Rio dos Sinos. São Leopoldo, Rio Grande do Sul, Brasil.
[5] Laboratório de Virologia, Departamento de Microbiologia, Imunologia e Parasitologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul.

A R T I C L E I N F O

Article history
Received 13 May 2020
Accepted 10 September 2020

Keywords:
Drug toxicity
Cell culture
MTT assay
Anesthesia
Analgesia

A B S T R A C T

Xylazine and acepromazine are drugs used exclusively in veterinary medicine. Xylazine is used as a sedative, analgesic, and tranquilizer while acepromazine is used as a sedative, pre-anesthetic, and anesthetic adjuvant. *In vitro* drug toxicity experimentation is essential to predict possible damage associated with treatment. This study was carried out to evaluate and compare the *in vitro* effects of acepromazine and xylazine on cell viability. Equine Dermis cells lines were used to examine different drug concentrations (0.02 mg/mL, 0.01 mg/mL, 0.005 mg/mL and 0.0025 mg/mL). An MTT assay was carried out to reveal cell viability. Both tested drugs reduced the viability of ED cells at 0.02 and 0.01 mg/mL. At 0.005 mg/mL, only acepromazine presented an effect. These results corroborate previous studies with xylazine. On the other hand, this is the first report about acepromazine and cell viability. Previous studies suggest that the mechanisms involved in reducing cell viability are apoptosis for xylazine and the activation of the autophagic pathway for acepromazine. Both mechanisms have been seen in other drugs of the same classes. These findings reveal that both acepromazine and xylazine cause concentration-dependent cytotoxicity *in vitro*. Future experiments could further elucidate the mechanisms by which this effect happens and thus circumvent the risk of potential tissue damage *in vivo*.

Palavras-chave:
Toxicidade de fármaco
Cultura de Células
Ensaio de MTT
Anestesia
Analgesia

R E S U M O

Xilazina e acepromazina são fármacos usados exclusivamente em medicina veterinária. A xilazina é usada como sedativo, analgésico e tranquilizante, enquanto a acepromazina é usada como sedativo, pré-anestésico e adjuvante anestésico. A experimentação de toxicidade de fármacos *in vitro* é essencial para prever possíveis danos associados ao tratamento. Nesse sentido, este estudo foi realizado com o objetivo de avaliar e comparar *in vitro* os efeitos da acepromazina e da xilazina na viabilidade celular. Células da linhagem Equine Dermis (ED) foram usadas para examinar diferentes concentrações de fármacos (0,02 mg/mL, 0,01 mg/mL, 0,005 mg/mL e 0,0025 mg/mL). O ensaio de MTT foi realizado para revelar a viabilidade celular. Ambos os fármacos testados reduziram a viabilidade das células ED em 0,02 e 0,01 mg/mL. A 0,005 mg/mL, apenas acepromazina apresentou efeito. Esses resultados corroboram estudos anteriores com xilazina. Por outro lado, este é o primeiro estudo sobre acepromazina e viabilidade celular. Estudos anteriores sugerem que os mecanismos envolvidos na redução da viabilidade celular são a apoptose para a xilazina, e a ativação da via autofágica para a acepromazina, ambos mecanismos observados em medicamentos das mesmas classes. Esses achados revelam que tanto a acepromazina quanto a xilazina causam citotoxicidade *in vitro* dependente da concentração. Expeimentos futuros podem elucidar ainda mais os mecanismos pelos quais esse efeito acontece e, assim, contornar o risco de possíveis danos aos tecidos.

* Corresponding author: giovana-araujo@agricultura.rs.gov.br

http://dx.doi.org/10.21708/avb.2020.14.4.9412

INTRODUCTION

Xylazine and acepromazine are drugs used exclusively in the clinical routine of veterinary medicine. Xylazine, an alpha$_2$ adrenergic receptor agonist (RANKIN, 2017), is used because of its sedative and analgesic properties in short-term procedures, in addition to inducing myorelaxation (MASSONE, 2011). It is delivered by subcutaneous (SC), intramuscular (IM) or intravenous (IV) routes (MASSONE, 2011) and goes through hepatic metabolism (TIBÚRCIO et al., 2014) and renal excretion (GROSS, 2003).

Acepromazine is a widely used drug in horses (CASTRO, 2003) and is currently the most widely used phenothiazine derivative in veterinary medicine (CORTOPASSI & FANTONI, 2010). It is used as a tranquilizer, sedative, pre-anesthetic, and anesthetic adjuvant (PAPICH, 2012). Its mechanism of action is mainly mediated by blocking dopaminergic receptors, although alpha$_1$ adrenergic, muscarinic, and histamine receptors are also affected (RANKIN, 2017). Administration can be oral (VO), subcutaneous (SC), intramuscular (IM), or intravenous (IV) (OLIVA & AGUIAR, 2015). Acepromazine is processed through hepatic biotransformation and its metabolites are eliminated via feces and urine (NEVES, 2013).

It is important to verify drug toxicity during *in vitro* experimentation because of the potential damage to the tissues of animals undergoing treatment. The cytotoxic effects of drugs, such as diazepam (ACOSTA & CHAPPELL, 1977; GAO et al., 2014), midazolam (WANG et al., 2018) detomidine (EDMONDS et al., 2016), triamcinolone (SYED et al., 2011), procaine (PEREZ-CASTRO et al., 2009), bupivacaine, ropivacaine and mepivacaine (BREU et al., 2013a), have already been described. Despite this, little is known about the effect of drugs such as xylazine and only a limited amount of studies have published about them (EDMONDS et al., 2016; MANCINI et al., 2017). As for acepromazine, no studies were found regarding its cytotoxic effect.

Cytotoxic assays such as these can be carried out *in vivo* but, to decrease the use of animal experimentation, numerous alternative methodologies have been adopted, such as tests in cell cultures, which can produce relevant *in vitro* results (HENRIQUES & SAMPAIO, 2002; BARROS, 2007). Likewise, assessments of cytotoxicity can be carried out on cells of different lineages and origins, according to each purpose, to achieve an approximation of the real effect of a given drug (ROGERO et al., 2003). Cell viability assays have been widely used to evaluate *in vitro* cytotoxic effects of different substances - including drugs (SAKAI, 2004; OLIVEIRA, 2015), plant extracts (ALVES et al., 2015) - and microorganisms, such as fungi (SANTOS, 2014), bacteria (TIBO, 2015) and viruses (SUMATHY et al, 2017).

Among the methods used to measure cytotoxicity on cell viability, the colorimetric 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay is one of the most widely used (ALVES & GUIMARÃES, 2010), due to its efficiency and reliability (BRITO et al., 2001). This method allows researchers to verify changes in mitochondrial metabolic activity in viable cell cultures (ABREU, 2008), making it possible to determine cell viability after a given treatment.

Given this possibility, the present study aims to evaluate the cytotoxic effect of acepromazine and xylazine using an MTT assay. Cell viability results after treatment with acepromazine were also compared with the previously described effects of xylazine (EDMONDS et al., 2016; MANCINI et al., 2017) on cell lines derived from equine dermis.

MATERIAL AND METHODS

Cell culture

Cells from the Equine Dermis cell line (ED ATCC® CCL-57 ™) were grown in 25cm^3 TPP® flasks, in an E-MEM medium (GIBCO®) supplemented with 10% fetal bovine serum (SFB) (GIBCO®) and 0.25% gentamicin (Gentatec®). The cells were maintained at 37 °C in a 5 % CO_2 incubator.

Drug preparation

Commercially available xylazine 2% (Sedanew® 2%, Vetnil) and acepromazine 0.2% (Acepran 0.2%, Vetnil) were acquired. Dilutions of 0.02 mg/ml, 0.01 mg/ml and 0.005 mg/ml were defined in accordance with Edmonds et al. (2016). Adaptations based on Garcia-Villar et al. (1992) included the addition of a 0.0025 mg/mL dilution to this experiment. E-MEM supplemented with SFB and gentamicin was used in all dilutions.

Evaluation of cell viability

ED cells were plated in 96-well tissue culture dishes in 4×10^4 cells/well. The plate was maintained at 37 °C in a humid environment with 5% CO_2. After, the medium was removed and the cells were exposed to four concentrations of xylazine or acepromazine in triplicate (0.02 mg/mL, 0.01 mg/mL, 0.005 mg/mL and 0.0025 mg/mL) and then incubated for 24 hours at 37°C in 5% CO_2. The integrity of the cells was verified with an inverted microscope after which the treatment was removed and 100 µL of MTT 0.5 mg/mL diluted in E-MEM was added, followed by 4 hours of incubation at 37 °C in a humid environment with 5% CO_2. Subsequently, the MTT was removed and 100 µL of DMSO (dimethylsulfoxide) was added to dissolve formazan crystals. The optical density (OD) was measured with a spectrophotometer (SpectraMax, Molecular Devices) at 540nm.

Effect of pH on cytotoxicity

Acepromazine and xylazine were diluted in a culture medium and the pHs of the solutions were determined with a pH meter (DM-22, Digimed).

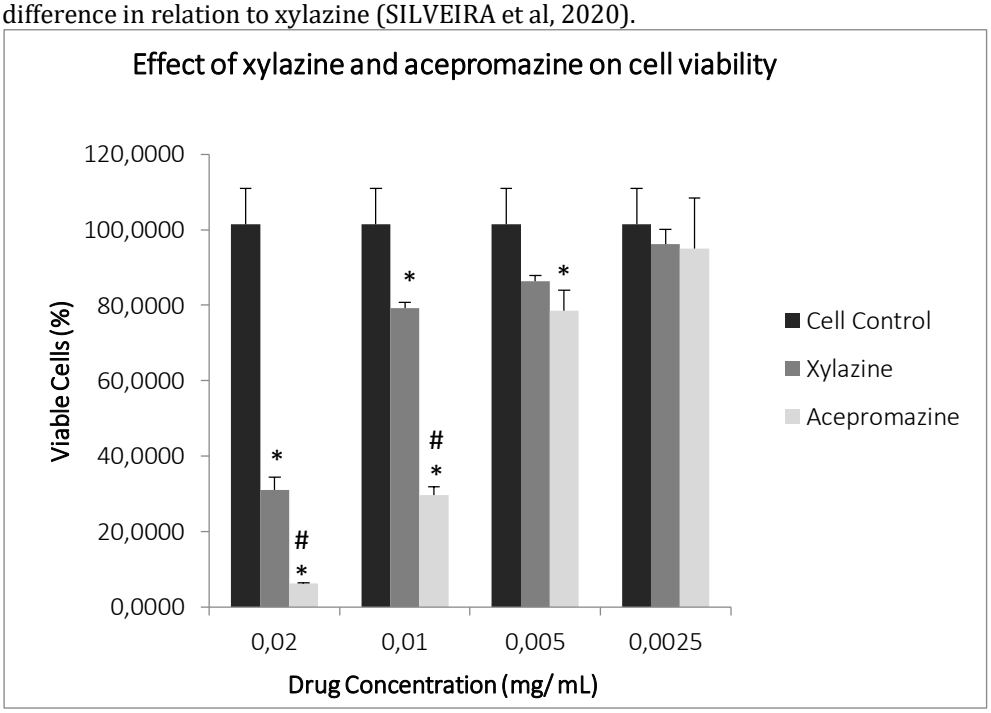
Statistical analysis

Data were obtained from experiments performed in triplicate. The mean and standard deviation for the MTT results were calculated. Data were expressed as mean ± standard deviation and analyzed by ANOVA, followed by the Tukey test when necessary, with statistical significance set at $p < 0.01$ (CALLEGARI-JACQUES, 2003). Statistical analysis was performed using VassarStats©.

RESULTS AND DISCUSSION

In this study, xylazine and acepromazine were evaluated for cytotoxic effects on ED cells using an MTT assay. Results indicated that these drugs reduced the viability of ED cells. When compared to control cells (100%), a xylazine dilution of 0.02 mg/mL preserved 30.61% of viable cells, 78.03% at 0.01 mg/mL, 85.11% at 0.005 mg/mL, and 94.68% at 0.0025 mg/mL. Regarding the acepromazine dilutions, 6.11% of viable cells were preserved at 0.02 mg/mL, 29.28% at 0.01mg/mL, 77.37% at 0.005 mg/mL and 93.54% at 0.0025 mg/mL, as shown in Figure 1.

Figure 1. Effect of xylazine and acepromazine on ED cell viability. Differences were considered significant when $p < 0.01$. Data were expressed as mean ± standard deviation. * indicates a significant difference in relation to controls; and # indicates a significant difference in relation to xylazine (SILVEIRA et al, 2020).



A decrease in the viability of the ED cell line was observed in a concentration-dependent manner for both drugs (xylazine and acepromazine). Besides what was found concerning drug concentration, the measured pHs of the xylazine (7.69) and acepromazine (7.76) solutions were close to the pH of the E-MEM (7.5) used in the cell cultures. Therefore, the pH values of the solutions used in this study should not have interfered with the results observed.

The results of the experiment corroborate those of other studies which showed that xylazine is toxic to thymocytes (CUPIĆ et al., 2003), splenocytes (CUPIĆ et al., 2001), chondrocytes (MANCINI et al., 2017), mesenchymal cells (EDMONDS et al., 2016) and endothelial cells (SILVA-TORRES et al., 2014). Mancini et al. (2017) assessed the cell morphology of chondrocytes treated with xylazine using cell cytometry and verified cell death due to apoptosis. This finding may be of clinical importance since, according to Edmonds et al. (2016), cell death in the region of application could contribute to a local inflammatory reaction. As such, the authors suggest that sedation with xylazine should be replaced with romifidine - a drug of the same class that did not significantly affect cell viability in that study. Alternatively, they recommend a combination of detomidine with butorphanol, which was also found to cause less cytotoxicity than xylazine.

Regarding acepromazine, so far there have been no reports of its effects on cell viability. Ours is the first study. Some authors have evaluated the role of mitochondrial permeability in cell death induced by phenothiazines in HTC cells (De Faria et al., 2015). Disruptions in mitochondrial function are crucial for regulating cell death, through cytochrome c release and apoptosis. This effect was also shown with

promethazine, a drug from the same pharmacologic group as acepromazine (Kemmerich et al., 2017; Medeiros et al., 2018). Previous results showed a 50% concentration-dependent decrease in cell viability from a concentration of 25 μM. This decrease was attributed to the activation of the autophagic pathway (Kemmerichet al., 2017), due to a decrease in mTOR protein expression and an increase in autophagic flux (Medeiros et al., 2018). Recently, Do Carmo et al. (2020) used computational simulations to certify if phenothiazines can compete with pro-apoptotic proteins for their binding-sites, which would explain the cytotoxic potential of phenothiazines. Taking all these data into account, the effects caused by acepromazine in ED cells were possibly triggered by the same mechanisms described for other phenothiazines. However, the relevance of this cytotoxic effect in clinical practice still needs to be described.

Cell death caused by the acidity of the drugs was excluded in this study, corroborating results from previous studies on chondrocyte lines (KARPIE and CHU, 2007; BREU et al., 2013a), mesenchymal cells (BREU et al., 2013b), and cells from human melanoma (KANG et al., 2015) exposed to lidocaine. In those studies, lidocaine was diluted in a buffering solution, a culture medium, or a saline buffered solution, which should have prevented the pH effect. We also avoided this effect in our study. However, it must be remembered that lidocaine belongs to the pharmacological group of local anesthetics (RANKIN et al., 2017). Therefore, the comparison of this drug with xylazine and acepromazine may be a limiting factor in the present study.

Results observed in this experiment may be not identical to those which occur *in vivo*, since damage may be limited by the abundant extracellular matrix that surrounds tissue (JUNQUEIRA & CARNEIRO, 2013). Similarly, it is not possible to observe an inflammatory response in cell cultures, since this is a complex response of organisms in the face of aggression; one which is impossible to mimic *in vitro* (MITCHELL et al., 2006). These reactions do not occur in cell cultures (ALVES & GUIMARÃES, 2010), although the cell line used in this study is of connective tissue origin. On the other hand, in addition to local painful processes, tissue injury due to medication can generate inflammatory reactions which may result in tissue necrosis (DUQUE & CHAGAS, 2009), especially following application failures or due to the individual sensitivity of each animal. However, this cannot be evaluated through *in vitro* experiments, although the known effects of phenothiazines upon cell death pathways could be inferred from *in vivo* tissue lesions. Despite indications that the tested concentrations cause cytotoxicity, additional studies are still needed to relate the present findings to medical clinical practice and to clarify the mechanisms by which acepromazine produces decreased cell viability.

CONCLUSIONS

In this experiment, we observed the cytotoxic activity of xylazine and acepromazine in ED cell lines occurring in a concentration-dependent manner. The pH of the drugs had no impact on the results. For pharmacology, this study is relevant since, until now, there has been no recognized effect of acepromazine on cell viability. In this pilot project, however, the possible cytotoxic activity of this drug *in vitro* has now been shown. Although this research addressed hypotheses about the effect of the tested drugs on cell viability *in vitro*, to confirm these findings and elucidate the mechanisms involved, more specific tests are needed.

REFERENCES

ABREU, Clarice Lima do Canto. Avaliação da citotoxicidade induzida por produtos cosméticos pelo método de quantificação de proteínas totais em células 3T3. Dissertação (Mestrado em Vigilância Sanitária) - Instituto Nacional de Controle de Qualidade em Saúde, Fundação Oswaldo Cruz, Rio de Janeiro. 2008. 122p. Disponível em <https://www.arca.fiocruz.br/bitstream/icict/9270/1/105.pdf>Acesso em 12 set. 2019.

ACOSTA, Daniel; CHAPPELL, Richard. Cardiotoxicity of diazepam in cultured heart cells. Toxicology, 1977. vol. 8. P 311-317.

ALVES, Emanuele Amorim; GUIMARÃES, Anna Christina Rosa. Cultivo Celular. Em: MOLINARO, Etelcia; CAPUTO, Luzia; AMENDOEIRA, Regina (Org.). Conceitos e métodos para formação de profissionais em laboratórios de saúde. Rio de Janeiro: EPSJV, 2010. p. 215-254. Disponível em: <http://www.fiocruz.br/ioc/media/vol_2%5B1%5D.pdf > Acesso em 26 set. 2019.

ALVES et al. AVALIAÇÃO DA CITOTOXICIDADE DE EXTRATOS DA PLANTA *Baccharis coridifolia.* Em: VIII MICTI - Mostra de Iniciação Científica do IFC - Instituto Federal Catarinense. Anais da V MIC - Mostra de Iniciação Científica. 2015.

BARROS, Kim Costa. Métodos alternativos para a substituição dos modelos animais na experimentação. Dissertação (Monografia) – Escola Politécnica de Saúde Joaquim Venâncio, Fundação Oswaldo Cruz, Rio de Janeiro. 2007. 53p. Disponível em: < http://www.hlog.epsjv.fiocruz.br/upload/monografia/20.pdf>Acesso em 22 set. 2019.

BREU, A. et al. Cytotoxicity of local anesthetics on human mesenchymal stem cells in vitro. Arthroscopy - Journal of Arthroscopic and Related Surgery, v. 29, n. 10, p. 1676–1684, 2013b.

BREU, A. et al. The cytotoxicity of bupivacaine, ropivacaine, and mepivacaine on human chondrocytes and cartilage. Anesthesiaand Analgesia, 2013a, v. 117, n. 2, p. 514–522.

BRITO et al. Lesão intestinal após isquemia-reperfusão: estudo comparativo usando sal tetrazólico (MTT) e histologia. Acta Cirúrgica Brasileira, São Paulo, jan./mar. 2001. v. 16, n. 1, p. 26-31. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-86502001000100005&lng=pt&nrm=iso>. Acesso em: 24 ago. 2019.

CALLEGARI-JACQUES, S. M. Bioestatística: princípios e aplicações. Porto Alegre: Artmed, 2003.

CASTILHO, J. G. et al. Padronização e aplicação da técnica de isolamento do vírus da raiva em células de Neuroblastoma de camundongo (N2a). Boletim Epidemiológico Paulista, São Paulo, v. 4, n. 47, p. 12-18, nov. 2007. Disponível em: < http://periodicos.ses.sp.bvs.br/pdf/bepa/v4n47/v4n47a02.pdf>. Acesso em 10 out. 2019.

CASTRO JUNIOR, Jarbas Francisco da Costa. Avaliação da influência da medicação pré-anestésica sobre os efeitos da anestesia geral intravenosa nos parâmetros endócrinos e metabólicos relacionados ao estresse em equinos. Tese (Doutorado) – Programa de Pós-Graduação em Medicina Veterinária, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre. 2004. Acta ScientiaeVeterinariae, 2018, v. 31, n. 2, p. 99. Disponível em: <https://seer.ufrgs.br/ActaScientiaeVeterinariae/article/view/16816/9876 > Acesso em: 02 out. 2019.

CHANG, Y. S. et al. Cytotoxicity of lidocaine or bupivacaine on corneal endothelial cells in a rabbit model. Cornea, 2006, v. 25, n. 5, p. 590–596.

CORTOPASSI, Silvia Renata Gaido; FANTONI, Denise Tabacchi. Medicação pré-anestésica. Em: FANTONI, Denise Tabacchi; CORTOPASSI, Silvia Renata Gaido. Anestesia em cães e gatos. 2. ed. São Paulo: Roca, 2010. p. 217-227.

CUPIĆ V. et al. Immunomodulatory effect of xylazine, an α2 adrenergic agonist, on rat spleen cells in culture. Journal of Neuroimmunology 2001, v. 113, p.19 - 29.

CUPIĆ V. et al. Xylazine, an α2-adrenergic agonist, induces apoptosis of rat thymocytes and a thymocytehybridoma line *in vitro*. Methods and Findings in Experimental and Clinical Pharmacology, 2003 v. 25, p. 05 - 10.

DE FARIA , P. A. et al. Cytotoxicity of phenothiazine derivatives associated with mitochondrial dysfunction: A structure-activity investigation. Toxicology, 2015, v. 330, p. 44-54.

DO CARMO, A. L. et al. Competition Between Phenothiazines and BH3 Peptide for the Binding Site of the Antiapoptotic BCL-2 Protein. Frontiers in Chemistry 2020, v. 8, p. 01-08.

DUQUE, Fernando Luis Vieira; CHAGAS, Carlos Alberto Araujo. Acidente por injeção medicamentosa no músculo deltoide: lesões locais e à distância, revisão de 32 casos. J Vasc Bras, v. 8, n 3. 2009.

EDMONDS, R. E. et al. Influence of commonly used pharmaceutical agents on equine bone marrow-derived mesenchymal stem cell viability. Equine Veterinary Journal, 2016, v. 49, n. 3, p. 352–357.

OLIVA, Valéria Nobre L.S.; AGUIAR, Antônio José de Araújo. Contenção Química: Contenção química de cães e gatos. Em: FEITOSA, Francisco Leydson F. Semiologia Veterinária: A Arte do Diagnóstico. Ed. Roca. 2008, p. 57.

GARCIA-VILLAR, R. et al. The pharmacokinetics of xylazine hydrochloride: an interspecific study. Journal of Veterinary Pharmacology and Therapeutics, 1981. v. 4, n. 2, p. 87–92.

GROSS, M. E. Tranquilizantes, agonistas alfa 2-adrenérgicos e agentes relacionados. Em: ADAMS, H. R. Farmacologia e Terapêutica em Veterinária. 8 ed. Rio de Janeiro: Guanabara Koogan, 2003. p. 249-284.

HENRIQUES, Maria das Graças Müller de Oliveira; SAMPAIO, André Luiz Franco. Alternativas para animais de laboratório: sistema *in vitro*. In: ANDRADE, Antenor; Pinto, Sergio Correia; OLIVEIRA, Rosilene Santos de (Org.) Animais de laboratório: criação e experimentação. Rio de Janeiro: Editora Fiocruz, 2002.

JUNQUEIRA, Luiz Carlos; CARNEIRO, José. Histologia básica: texto e atlas. 12.ed. Rio de Janeiro: Guanabara Koogan, 2013. p. 90.

KANG, Ding-Kun; ZHAO, Li-Yan;WANG, Hong-Li. Efeitos citotóxicos de anestesia local com lidocaína/ropivacaína em linhagens celulares de melanoma humano. Brazilian Journal of Anesthesiology, 2016. v. 66, n. 6, p. 594–602.

KARPIE, John C.; CHU, Constance R. Lidocaine exhibits dose- and time-dependent cytotoxic effects on bovine articular chondrocytes in vitro. American Journal of Sports Medicine, 2007. v. 35, n. 10, p. 1622–1627.

KEMMERICH, Karoline Kristina; RODRIGUES, Tiago. Efeitos de compostos derivados da prometazina: estudo de mecanismos mitocondriais relacionados à citotoxicidade. Em: XX Congresso de Iniciação Científica da UMC, 2017, Mogi das Cruzes. Livro de Resumos, 2017.

LOWRY, Richard. Vassarstats©, 2019. Disponível em: <http://vassarstats.net/> Acesso em: 16 set. 2019.

MANCINI, F. et al. Effects of xylazine and dexmedetomidine on equine articular chondrocytes in vitro. VeterinaryAnaesthesiaand Analgesia, 2017. v. 44, n. 2, p. 295–308.

MASSONE, Flávio. Anestesiologia veterinária: farmacologia e técnicas. 6. ed., Rio de Janeiro, Guanabara Koogan, 2011. 571p.

MEDEIROS et al. Autophagy-mediated cell death in leukemia cells induced by promethazine. Em: XIX Congress of The Brazilian Society for Cell Biology, 2018, São Paulo. Livro de Resumos, 2018.

MITCHELL, RN; KUMAR, V; ABBAS, AK; FAUSTO, N. Inflamação aguda e crônica. Em: Robbins &Cotran, Fundamentos de Patologia. 7ª ed. Rio de Janeiro: Elsevier, 2006. p. 29-44.

NEVES et al. Avaliação do ensaio imediato para a pesquisa de compostos fenotiazínicosna urina utilizando o reagente de forrest modificado. Revista Brasileira de Toxicologia, 2003. v. 16, n. 1, p. 122, suppl.

OLIVEIRA, Maria Eduarda Ismerio Moreira de. Avaliação do Efeito Citotóxico de Piranonaftoquinonas Inibidoras de DNA Topoisomerases Sobre Células de Leucemia. 2015. 100p. Dissertação (Mestrado em Biologia Celular e Molecular) – Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro.

PAPICH, Mark G. Manual Saunders de Terapia Veterinária – Pequenos e Grandes Animais. Rio de Janeiro. Editora Elsevier. 3ª ed. 2012. p. 268.

PARK, J. et al. Comparison of the cytotoxic effects of bupivacaine, lidocaine, and mepivacaine in equine articular chondrocytes. Veterinary Anaesthesia and Analgesia, 2011. v. 38, n. 2, p. 127–133.

PEREZ-CASTRO, R. et al. Cytotoxicity of local anesthetics in human neuronal cells. Anesthesia and Analgesia, 2009. v. 108, n. 3, p. 997–1007.

PICIOLI, A. et al. O uso da acepromazina, dexmedetomidina e xilazina na sedação em cães : alterações hematológicas e bioquímicas. Revista Brasileira de Ciência Veterinária, 2013. v. 20, n. 1, p. 13–19.

PICOLI, T. et al. Toxicidade E Eficiência Do Dimetilsulfóxido (DMSO) No Congelamento De Células Madin-DarbyBovineKidney (MDBK). Science And Animal Health, 2015. v. 3, n. 2, p. 159.

RANKIN, D.C. Sedativos e tranquilizantes. Em: TRANQUILLI, W..J.; THURMON, J.C.; GRIMM, K.A. Lumb& Jones Anestesiologia e analgesia veterinária. 5.ed. Rio de Janeiro: Roca, 2017. p.188-198.

RIBEIRO, G. et al. Efeitos de detomidina e xilazina intravenosa sobre as variáveis basais e respostas comportamentais em bovinos. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia, 2012. v. 64, n. 6, p. 1411–1417.

ROGERO, S. O. et al. Teste in vitro de citotoxicidade: estudo comparativo entre duas metodologias. MaterialsResearch, 2003. v. 6, n. 3, p. 317–320.

SAKAI, Mônica. Efeitos do diazepam sobre o crescimento tumoral e imunidade de animais portadores do Tumor Ascítico de Ehrlich. 2004. 129p. Dissertação (Mestrado em Patologia) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo.

SANTOS, Beatriz TaynaraAraúdo dos. Estudo da atividade dos extratos de plantas e fungos endofíticos isolados do cerrado brasileiro em linhagem celular de feocromocitoma.2014. 74p. Dissertação (Mestrado em Ciências Farmacêuticas) – Universidade de Brasília, Brasília.

SILVA-TORRES et al. Toxic effects of xylazine on endothelial cells in combination with cocaine and 6-monoacetylmorphine. Toxicol*In Vitro*. 2014. p. 1312 - 1319.

SUMATHY, K. et al. Protective efficacy of Zika vaccine in AG129 mouse model.Scientific Reports, 2017. v. 7, n. December 2016, p. 1–9.

SYED, H.M., et al., Bupivacaine and triamcinolone may be toxic to human chondrocytes: a pilot study. ClinOrthopRelat Res, 2011. 469(10): p. 2941-7.

TIBO, Luiz Henrique Soares. Caracterização de efeito causado por citotoxina secretada por *Escherichia coli*associada à sepse em células endoteliais. 2015. 71p. Dissertação (Mestrado em Genética e Biologia Molecular) – Universidade Estadual de Campinas, Campinas, São Paulo.

TIBURCIO, M. et al. Acepromazina, detomidina ou xilazina na sedação em equinos: efeitos hematológicos e bioquímicos. Revista Acadêmica Ciência Animal, 2014. v. 12, n. 1, p. 35.

WANG, C., et al., Midazolam and Dexmedetomidine Affect Neuroglioma and Lung Carcinoma Cell Biology In Vitro and In Vivo. Anesthesiology. 2018;129(5):1000-1014.