The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

Unveiling the neurotoxicity of mitoxantrone: oxidative stress, apoptosis, and autophagy in the brain of adult CD-1 mice

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

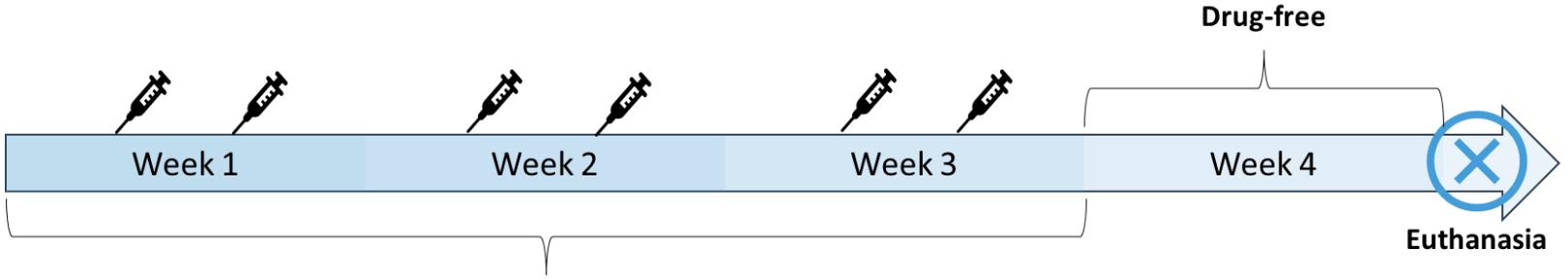
pharmaceuticals MDPI

Ana Dias-Carvalho[1, 2*], Mariana Ferreira[1,2,3], Ana Reis-Mendes[1,2], Rita Ferreira[3], Eduarda Fernandes[4], Susana Isabel Sá[5,6], João Paulo Capela[1,2,7], Félix Carvalho[1,2], Vera Marisa Costa[1,2]

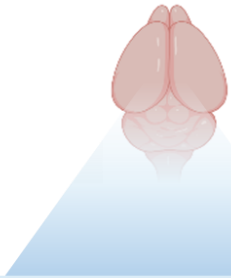
[1]Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal;
[2]UCIBIO - Applied Molecular Biosciences Unit, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal;
[3]LAQV/REQUIMTE, Chemistry Department, University of Aveiro, Aveiro, Portugal;
[4]LAQV/REQUIMTE, University of Porto, Porto, Portugal;
[5]Unit of Anatomy, Department of Biomedicine, Faculty of Medicine, University of Porto, Porto, Portugal;
[6]CINTESIS@RISE, Unit of Anatomy, Department of Biomedicine, Faculty of Medicine, University of Porto, Porto, Portugal;
[7]FP-I3ID, Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Porto, Portugal.

* Corresponding author: arcdc97@gmail.com

Unveiling the neurotoxicity of mitoxantrone: oxidative stress, apoptosis, and autophagy in the brain of adult CD-1 mice

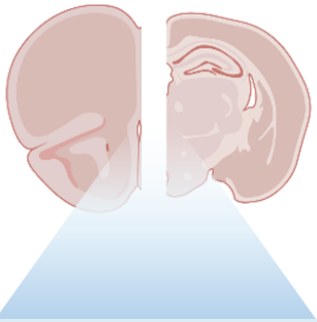


Total cumulative dose of **6 mg/kg Mitoxantrone**



Oxidative stress
Impaired oxidative metabolism
Autophagy
Loss of post synaptic integrity

Whole brain



Decreased volume of Hippocampal formation
Apoptosis
Hyperphosphorylation of pTau

Prefrontal cortex and Hippocampal formation

Abstract: Mitoxantrone (MTX) is a topoisomerase II inhibitor with anticancer and immunomodulatory properties, however its neurotoxicity is poorly understood. Our work aimed to evaluate the neurotoxicity of a clinically relevant dose of MTX in adult mice.

Three-month-old CD-1 male mice received bi-weekly administrations of MTX for 3 weeks, until they achieved a total cumulative dose of 6 mg/kg. They were sacrificed one week later. Biomarkers of oxidative stress, neuronal damage, apoptosis, and autophagy were analysed in whole brain, whereas brain sections were used for analysis of the hippocampal formation (HF) and prefrontal cortex (PFC).

Our results demonstrated that MTX induced redox imbalance in the whole brain, namely an increase in endothelial nitric oxide synthase and reduced manganese superoxide dismutase content. Brain oxidative metabolism was altered as seen by diminished subunit β adenosine triphosphate synthase content. MTX increased autophagic microtubule-associated protein light chain 3 II and decreased postsynaptic density protein 95. Regarding regional brain analysis, a reduction in volume was observed in the dentate gyrus and CA1 region of the HF. Total number of glial fibrillary acid protein immunoreactive astrocytes increased in all regions of the HF except in the DG, suggesting extensive astrogliosis. The apoptotic marker Bax increased in the PFC and CA3 region, whereas p53 decreased in all areas evaluated. In the PFC, MTX caused hyperphosphorylation of Tau.

MTX disrupts several pathways in the brain of adult CD-1 mice in a clinically relevant dose, which can lead to cognitive impairment, but further studies are needed to evaluate the putative consequences.

Keywords: Chemobrain; chemotherapy; mitoxantrone; neurotoxicity

Introduction

Mitoxantrone (MTX)

Inhibits DNA and RNA synthesis via DNA intercalation and **topoisomerase II inhibition.**

Treatment of several types of cancers like **acute leukaemia**, **prostate cancer** and advanced metastatic **breast cancer.**

Treatment of **worsening relapsing-remitting** or **secondary-progressive multiple sclerosis.**

The total lifetime cumulative dose of **140 mg/m^2 should not be exceeded**.

Introduction

‘Chemobrain’

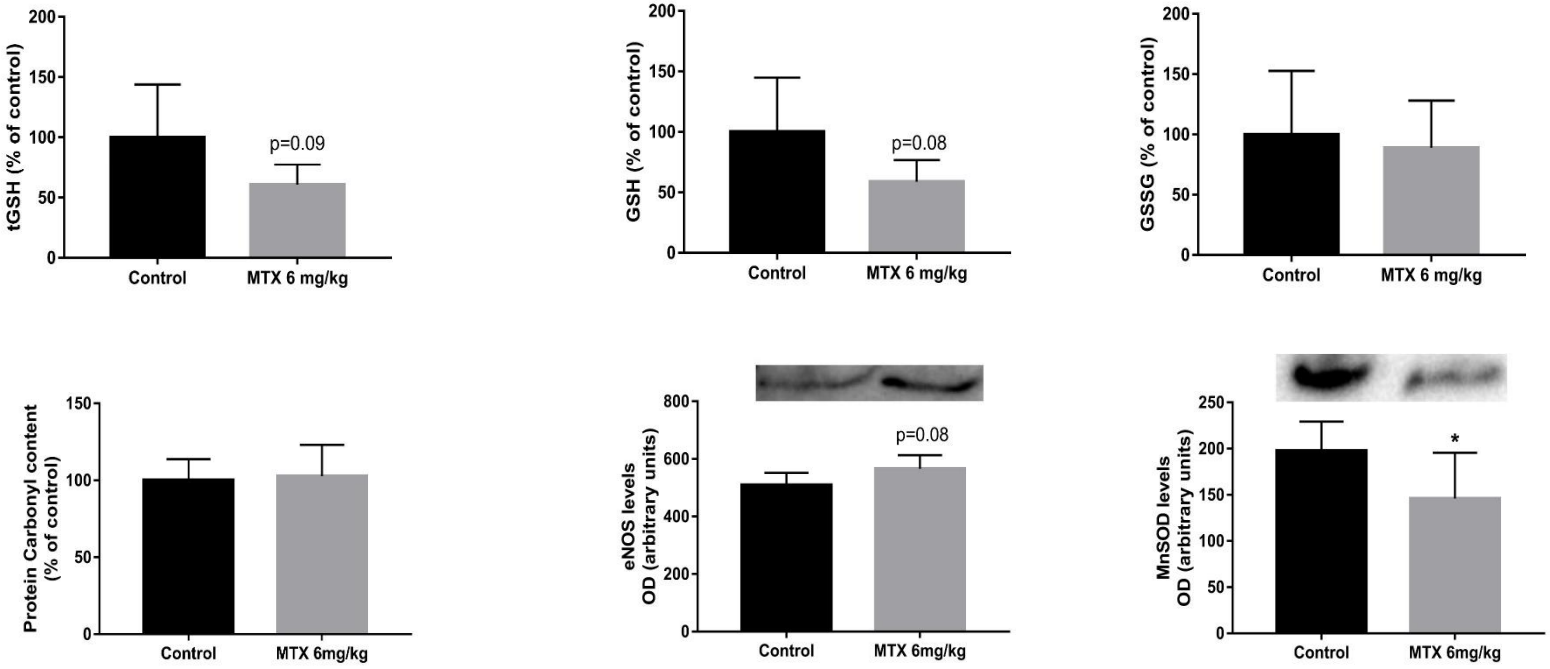
The cognitive deficit effects caused by chemotherapy in the long term.

It could affect between 28% to 75% of the patients submitted to chemotherapy.

Through direct or indirect mechanisms, the chemotherapeutics can overcome the protection of the blood brain barrier (BBB) and damage the brain.

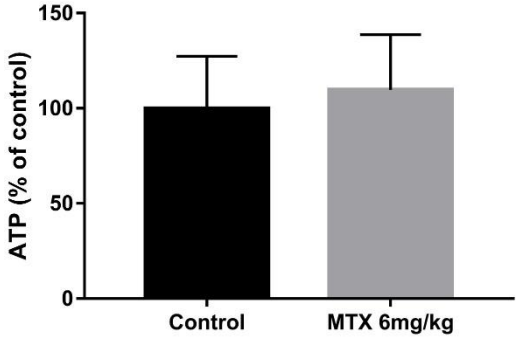
Results and discussion

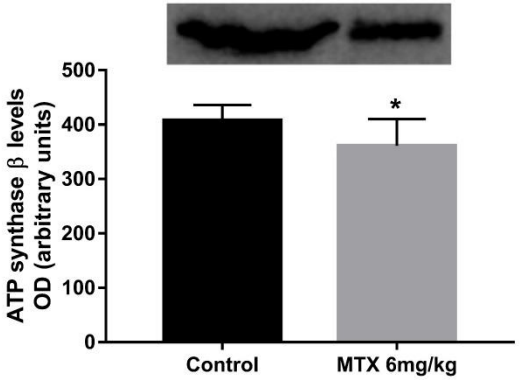
MTX evoked redox imbalance in the brain

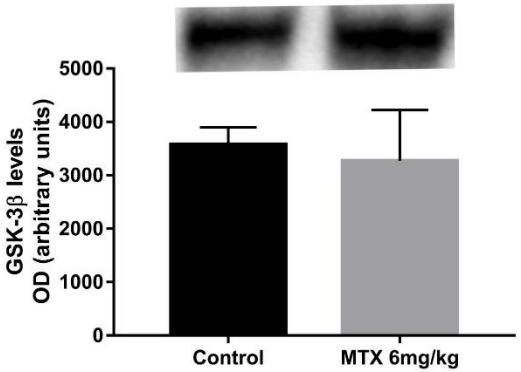


Available at: Dias-Carvalho, A., Ferreira, M., Reis-Mendes, A. et al. Chemobrain: mitoxantrone-induced oxidative stress, apoptotic and autophagic neuronal death in adult CD-1 mice. Arch Toxicol 96, 1767–1782 (2022). https://doi.org/10.1007/s00204-022-03261-x

MTX decreased ATP synthase β expression without meaningful changes in ATP levels

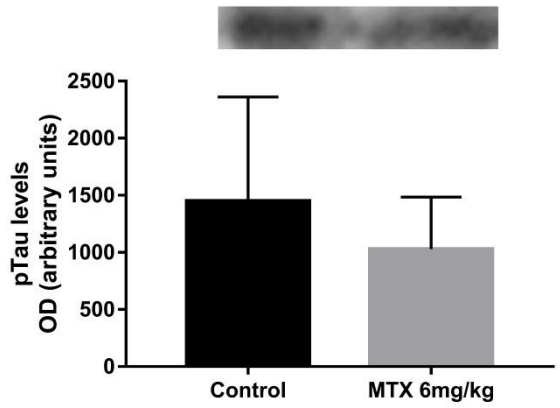


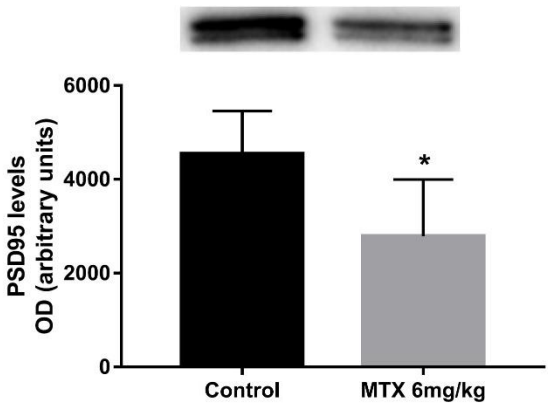


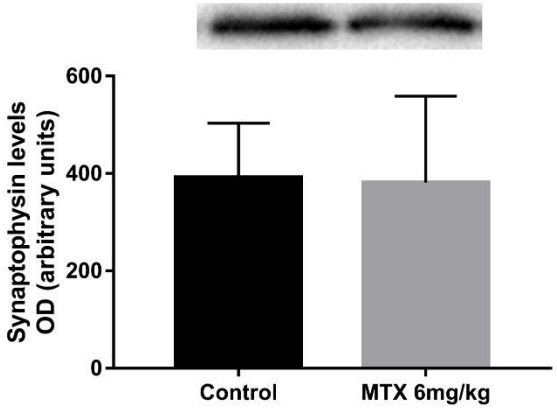


Available at: Dias-Carvalho, A., Ferreira, M., Reis-Mendes, A. et al. Chemobrain: mitoxantrone-induced oxidative stress, apoptotic and autophagic neuronal death in adult CD-1 mice. Arch Toxicol 96, 1767–1782 (2022). https://doi.org/10.1007/s00204-022-03261-x

MTX decreased the expression of the postsynaptic protein PSD95, but it did not change the levels of pTau or synaptophysin

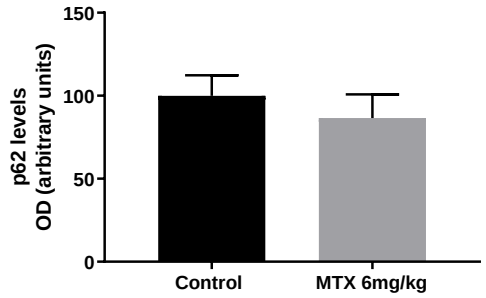


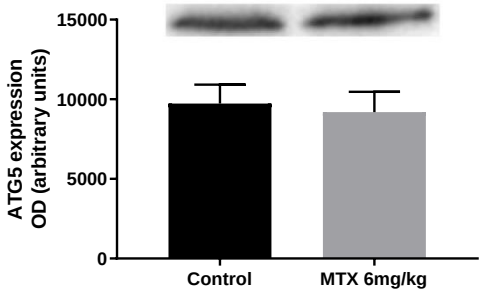


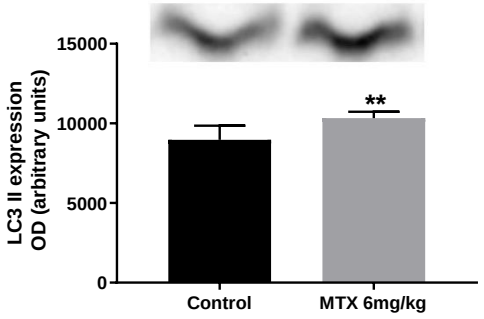


Available at: Dias-Carvalho, A., Ferreira, M., Reis-Mendes, A. et al. Chemobrain: mitoxantrone-induced oxidative stress, apoptotic and autophagic neuronal death in adult CD-1 mice. Arch Toxicol 96, 1767–1782 (2022). https://doi.org/10.1007/s00204-022-03261-x

MTX increased expression of autophagic protein LC3-II, with a tendency for decreased p62 expression with no changes in ATG5 levels

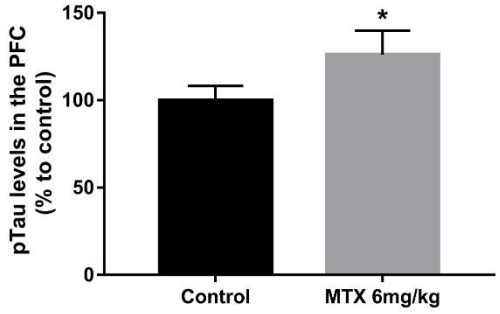


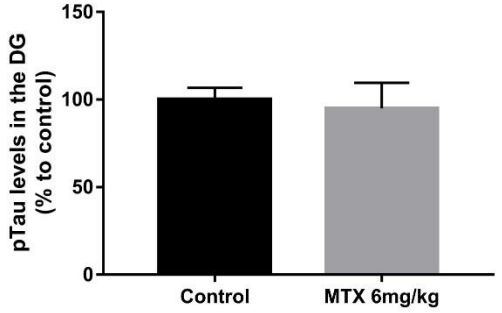


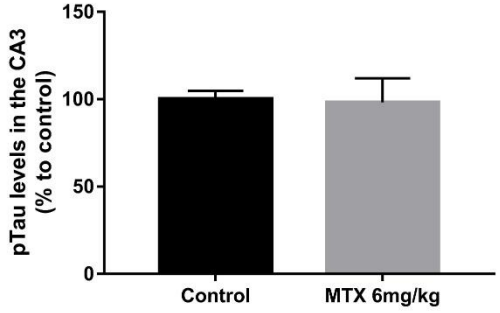


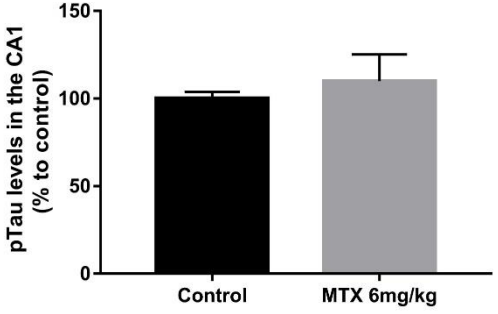
Available at: Dias-Carvalho, A., Ferreira, M., Reis-Mendes, A. et al. Chemobrain: mitoxantrone-induced oxidative stress, apoptotic and autophagic neuronal death in adult CD-1 mice. Arch Toxicol 96, 1767–1782 (2022). https://doi.org/10.1007/s00204-022-03261-x

MTX increased pTau in the PFC area



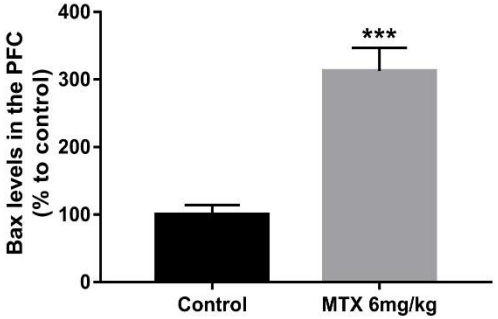


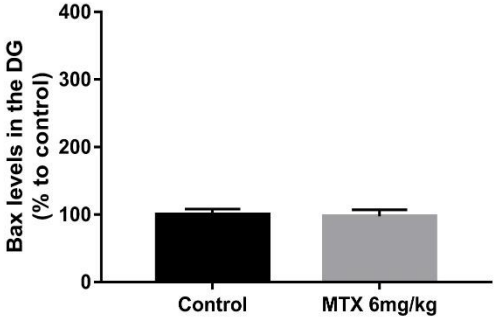


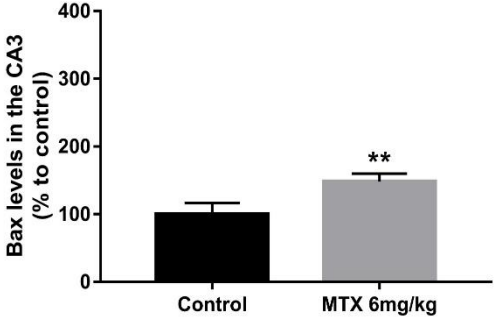


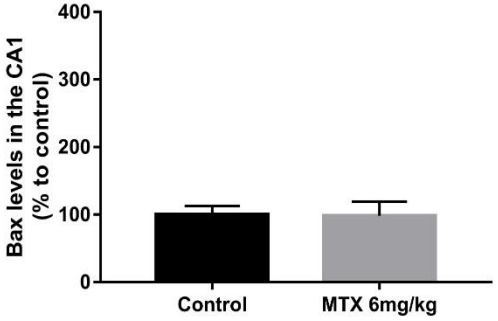
Available at: Dias-Carvalho, A., Ferreira, M., Reis-Mendes, A. et al. Chemobrain: mitoxantrone-induced oxidative stress, apoptotic and autophagic neuronal death in adult CD-1 mice. Arch Toxicol 96, 1767–1782 (2022). https://doi.org/10.1007/s00204-022-03261-x

MTX increased Bax expression in both PFC and CA3 region of the HF



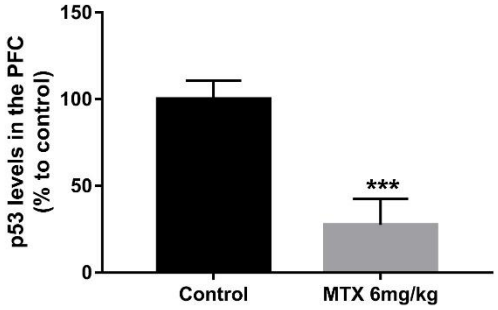


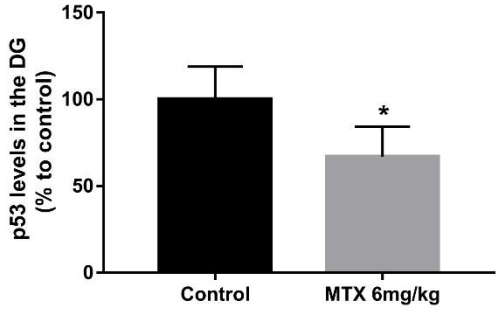


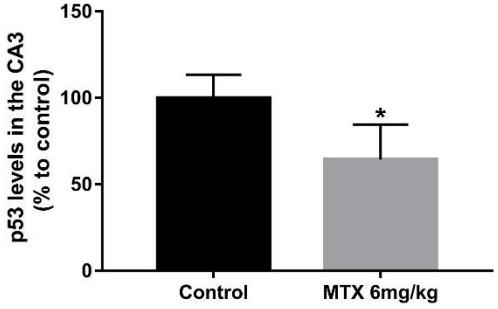


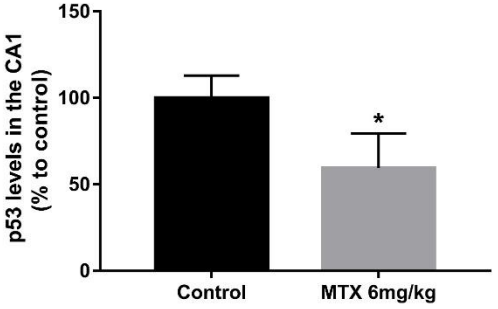
Available at: Dias-Carvalho, A., Ferreira, M., Reis-Mendes, A. et al. Chemobrain: mitoxantrone-induced oxidative stress, apoptotic and autophagic neuronal death in adult CD-1 mice. Arch Toxicol 96, 1767–1782 (2022). https://doi.org/10.1007/s00204-022-03261-x

MTX-treated mice presented a decreased expression of p53 in all brain areas evaluated



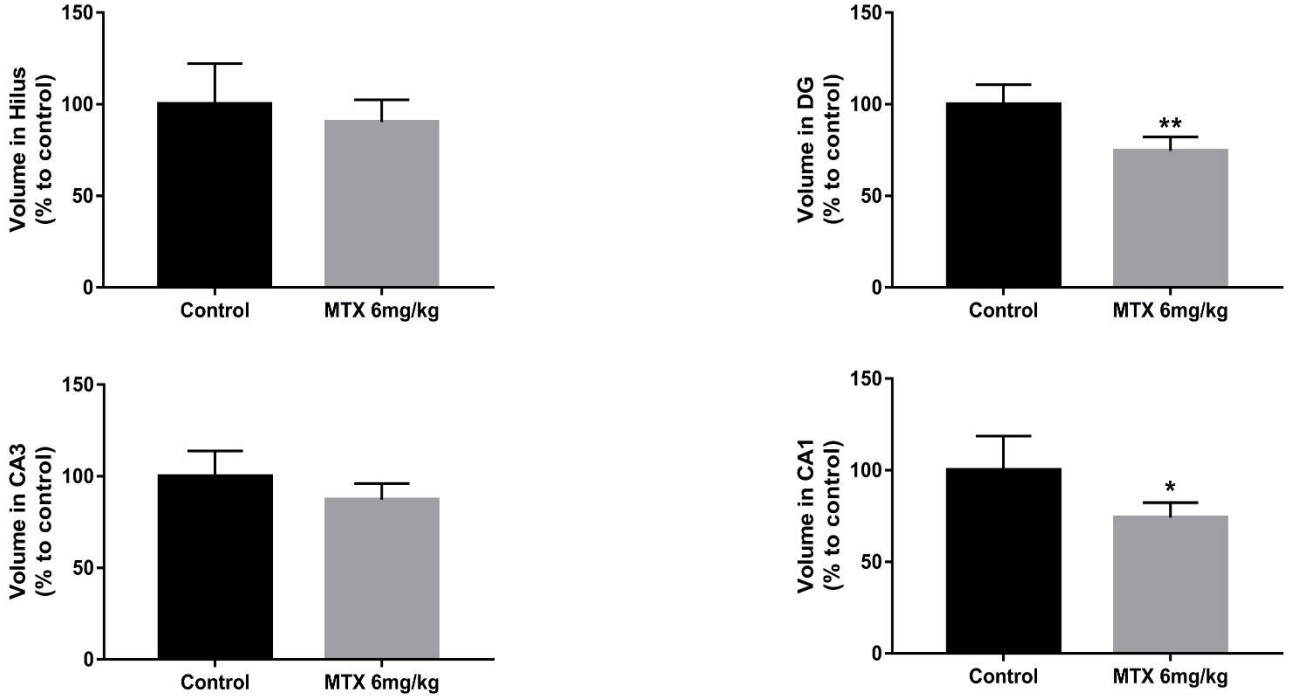






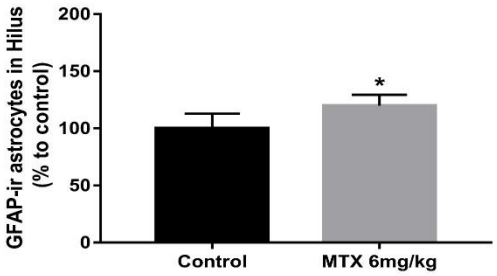
Available at: Dias-Carvalho, A., Ferreira, M., Reis-Mendes, A. et al. Chemobrain: mitoxantrone-induced oxidative stress, apoptotic and autophagic neuronal death in adult CD-1 mice. Arch Toxicol 96, 1767–1782 (2022). https://doi.org/10.1007/s00204-022-03261-x

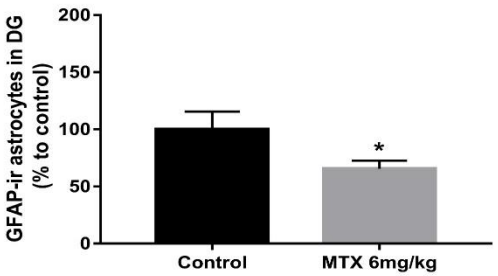
MTX-treated mice presented a decreased in HF volume in DG and CA1 regions

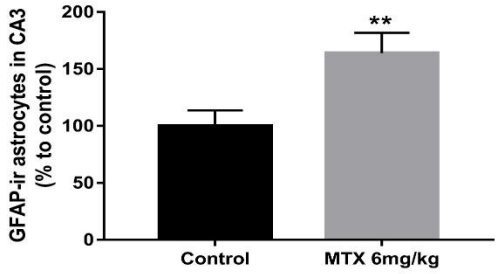


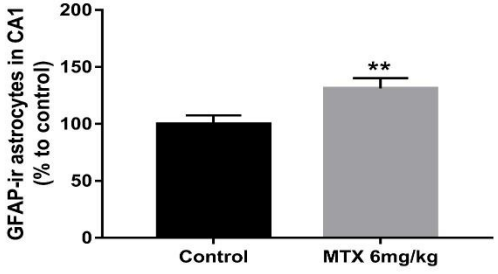
Available at: Dias-Carvalho, A., Ferreira, M., Reis-Mendes, A. et al. Chemobrain: mitoxantrone-induced oxidative stress, apoptotic and autophagic neuronal death in adult CD-1 mice. Arch Toxicol 96, 1767–1782 (2022). https://doi.org/10.1007/s00204-022-03261-x

MTX-treated animals showed an increase in GFAP-ir astrocytes in three HF regions









Available at: Dias-Carvalho, A., Ferreira, M., Reis-Mendes, A. et al. Chemobrain: mitoxantrone-induced oxidative stress, apoptotic and autophagic neuronal death in adult CD-1 mice. Arch Toxicol 96, 1767–1782 (2022). https://doi.org/10.1007/s00204-022-03261-x

Conclusions

- MTX caused **oxidative stress**, **alterations in brain metabolism**, **increased autophagy** and **decreased neuronal integrity**.

- In the HF and PFC, MTX caused a **decrease in volume** and **triggered astrogliosis** in HF, increased **apoptotic neuronal death** in the PFC and HF and caused **hyperphosphorylation of Tau** in the PFC.

MTX can overcome the protection of the BBB and harm the brain with putative consequences that need to be clarified

Acknowledgments

This work is financed by national funds from Fundação para a Ciência e a Tecnologia (FCT), I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences (UCIBIO) and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB and through the project EXPL/MEDFAR/0203/2021. A. Dias-Carvalho acknowledges FCT and UCIBIO for her PhD grant (UI/BD/151318/2021). V.M.C acknowledges FCT for her grant (SFRH/BPD/110001/2015) that was funded by national funds through FCT under the Norma Transitória – DL57/2016/CP1334/CT0006. A.R.-M. acknowledges FCT for her grant SFRH/BD/129359/2017.