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Modulation of nitro-oxidative parameters in mice administered with very low doses of the iridoid plumieride

Modulação de parâmetros nitro-oxidativos em camundongos tratados com doses baixíssimas de plumierídeo

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ABCTRACT

Our group has been investigating the anti-inflammatory and antidepressant-like effects of plumieride (PLU 0.5, 1, 2 µg/Kg). However, there is no record in the literature about the in vivo antioxidant activity of PLU at very low doses, which could be linked with pharmacology effects. Here, we administered mice with vehicle and PLU 0.5, 1, or 2 µg/Kg for 7 days. Subsequently, we evaluated the modulation of lipoperoxidation, nitrite, protein carbonylation, and non-protein thiols triggered by PLU in mice's brain, plasma, liver, and kidneys. Our results support, at least in part, the pharmacological potential that has been attributed to iridoid.

Key words: Plumieride; iridoid; *Allamanda cathartica*; oxidative stress.

RESUMO

Nosso grupo de pesquisa tem investigado os efeitos anti-inflamatório e tipo-antidepressivo do plumierídeo (PLU – 0,5, 1 e 2 µg/Kg). Contudo, não há evidências na literatura científica quanto a atividade antioxidante do PLU quando usado em doses extremamente baixas, que podem ser relacionados aos seus efeitos farmacológicos. Neste estudo, foi administrado PLU (0,5, 1 e 2 µg/Kg) à camundongos durante 7 dias. Posteriormente, nós avaliados a capacidade do PLU em modular a lipoperoxidação, nitritos, carbonilação de proteínas e tióis não-proteicos no cérebro, plasma, fígado e rins dos camundongos. Nossos resultados fundamentam, pelo menos em parte, o potencial farmacológico que tem sido atribuído ao iridoide.

Palavras-chave: Plumierídeo; iridoide; *Allamanda cathartica*; estresse oxidativo.

1 INTRODUCTION

Iridoids are terpene compounds from plant' secondary metabolism and have shown pharmacological potential as hypolipidemic, antiviral, antibacterial, hypoglycemic, and anti-inflammatory (HUSSAIN et al., 2018; LIU et al., 2017; PANKOKE et al., 2013; XIA et al., 2018). Plumieride (PLU), a characteristic representative of iridoids, is a molecule isolated from *Allamanda cathartica* flowers and plants of genus *Plumeria* and *Plantago.* The antinociceptive, anti-inflammatory, hepatoprotective, and immunostimulatory effects are already attributed to PLU (BOEING et al., 2018; SINGH et al., 2014; SINGH et al., 2017). Also, plumieride did not interfere with the locomotor capacity of mice after acute administration and did not exert anticonvulsant effect (DALMAGRO et al., 2019).

Noteworthy, a recent study published by our research group indicate that PLU was responsible for antidepressant-like effect after an acute intraperitoneal administration in female mice evaluated in Forced Swimming Test (FST), at 1 µg/Kg; besides its low toxicity and high solubility in water (BONOMINI et al., 2017; SINGH et al., 2017). However, evidence has shown that anti-inflammatory and antidepressant effects may be linked to the antioxidant capacity of some compounds (SLAVICH; IRWIN, 2014). Nonetheless, there are no reports in the literature about the *in vivo* antioxidant capacity of PLU after the administration of very low doses.

Therefore, this research aimed to investigate the ability to modulate the lipid peroxidation, nitrite level, carbonyl protein content, and non-proteic thiols group triggered by PLU in the brain, plasma, liver, and kidney of mice treated for 7 days.

2 METHODOLOGY

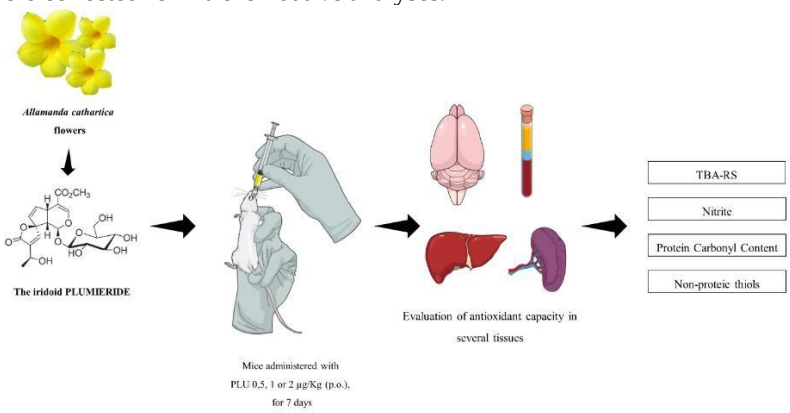
2.1 PLANT MATERIAL AND ISOLATION OF PLUMIERIDE

PLU was isolated from *Allamanda cathartica* flowers, and its purity (>98%) was verified by HPLC and NMR analysis, according to our previous studies (BONOMINI et al., 2017; MULLER et al., 2015). PLU was dissolved only in distilled water for oral administration.

2.2 ANIMALS AND TREATMENTS

Biochemical analyzes were performed with adult female *Swiss* mice weighing 25-30 g, obtained from UNIVALI Central Animal Facility. The animals were kept in the sectoral vivarium with food and water *ad libitum*, under a climate-controlled room at 22 ± 2 ºC and light/dark (12:12) cycle – lights on at 6:30 a.m. CEUA/UNIVALI approved all procedures performed in this research – 035/2016. Vehicle (destilled water) and PLU 0.5, 1 and 2 µg/Kg were administered for 7 days by gavage. 24 hours from the last administration, mice were euthanized and quickly collected the brain, plasma, liver, and kidneys. The tissues were homogenized in phosphate buffer solution (DALMAGRO et al., 2017). Whole blood was also collected, taken to the centrifugation for separation of plasma (3000 x g, 10 min). All procedures conducted in the research are represented in figure 1.

Figure 1. Schematic representation of the procedures conducted in the present research: The mice were treated with PLU (0.5, 1, 2 µg/kg) for 7 days. Animals were euthanized 24 hours after the last gavage, and brain, plasma, liver and kidneys were collected for nitro-oxidative analyses.



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2.3 LIPID PEROXIDATION ASSAY (TBA-RS)

An aliquot of sample or plasma was mixed with thiobarbituric acid (TBA) according to the reaction conditions described by Ohkawa et al. (1979). This method is based on the quantification of malondialdehyde (MDA) at 535 nm. Results of thiobarbituric acid reactive substances (TBA-RS) are expressed as nmol/mg protein.

2.4 EVALUATION OF NITRITE LEVELS

An aliquot of the homogenate or plasma (50 µL) was mixed with 100 µL of the Griess' Reagent (1879), kept at room temperature for 10 min and measured the absorbance at 525 nm. A previous curve with various concentrations of $NaNO_2$ was established to quantify the nitrite levels, and the results were expressed in µmol/mg protein.

2.5 PROTEIN CARBONYL (PC) CONTENT

Protein carbonyl content was estimated by mixing homogenate or plasma with 10 mM of DNPH (2,4-dinitro-phenylhydrazine) in acid medium (HCl 2M) and guanidine 6M. Protein carbonyl content was expressed in nmol/mg protein after spectrophotometric reading at 370 nm (REZNICK; PACKER, 1994).

2.6 DETERMINATION OF NON-PROTEIN THIOL GROUPS (NPSH)

Endogenous antioxidant defenses were quantified in the samples by lysing them with 0.4M Tris-HCl buffer (pH 8.9) and exposed to the Ellman's reagent. The absorbances were determined at 405 nm, and results were expressed as nmol of NPSH/mg protein (ELLMAN, 1959).

2.7 TOTAL PROTEIN CONTENT

Bovine albumin was used as a standard for protein quantification in accordance to Lowry et al. (1951).

2.8 STATISTICAL ANALYSIS

Obtained data were analyzed through one-way analysis of variance – ANOVA - followed by Tukey's test, supported by GraphPad Prism software version 7.0. The results are presented as mean ± S.D. and were considered significant when $p < 0.05$.

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3 RESULTS AND DISCUSSION

The results obtained from the analysis of the brain indicate that PLU 1 and PLU 2 increased lipid peroxidation ($p<0.05$; $p<0.0001$), as depicted in figure 2. PLU 2 also induced higher nitrite production ($p<0.01$). However, all doses stimulated the increase of NPSH ($p<0.05$; $p<0.001$; $p<0.0001$, respectively). The brain is predominantly composed of lipids, has a high metabolic rate, and consequently, it is more subject to oxidative stress (MAES et al., 2011). One limitation of our study was the use of the whole brain for analysis (DALMAGRO et al., 2017); but it is essential to note that PLU was able to stimulate an critical endogenous antioxidant defense. Some researchers have reported the increase in NPSH as essential for antidepressant and anxiolytic effects of specific molecules (DONATO et al., 2013; GIBSON et al., 2012).

Figure 2. Effects of vehicle (distilled water, p.o.) or PLU (0.5, 1 or 2 µg/kg, p.o.) on the oxidative parameters measured in the mice´s brain after administration for 7 days: **(A)** Lipid peroxidation (TBA-RS); **(B)** Nitrite level; **(C)** Protein carbonyl content; **(D)** Non-protein thiols groups. Results are expressed as means ± S.D. – n=5-6. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 compared to vehicle-treated control.

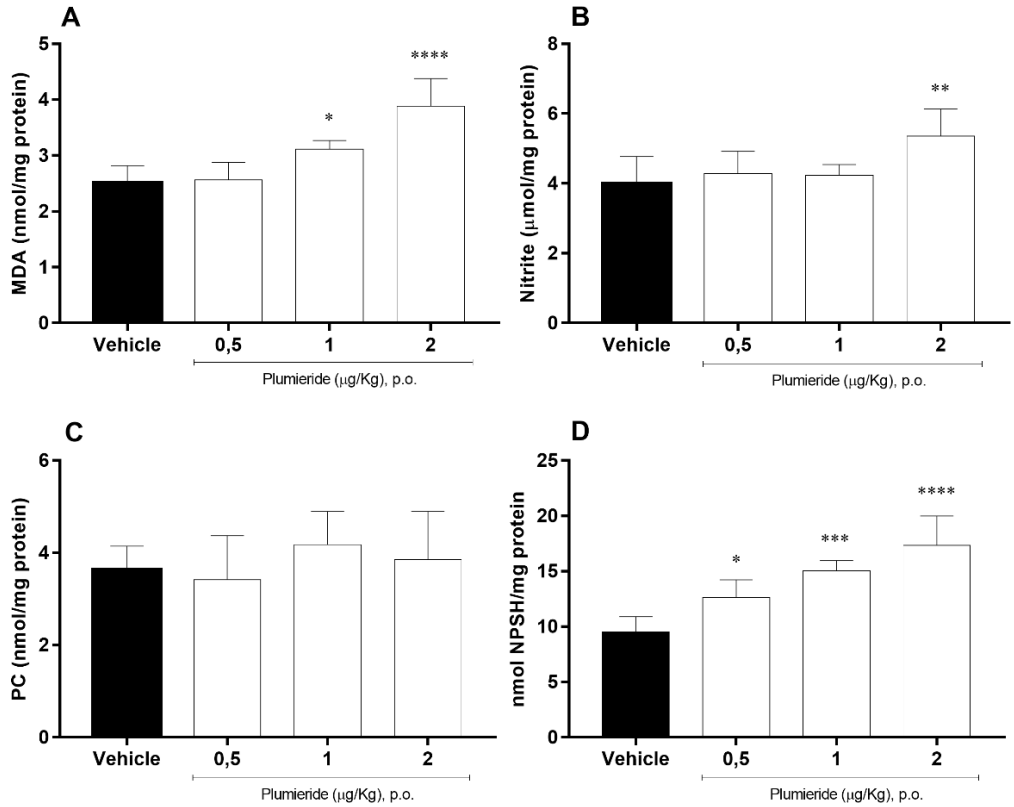
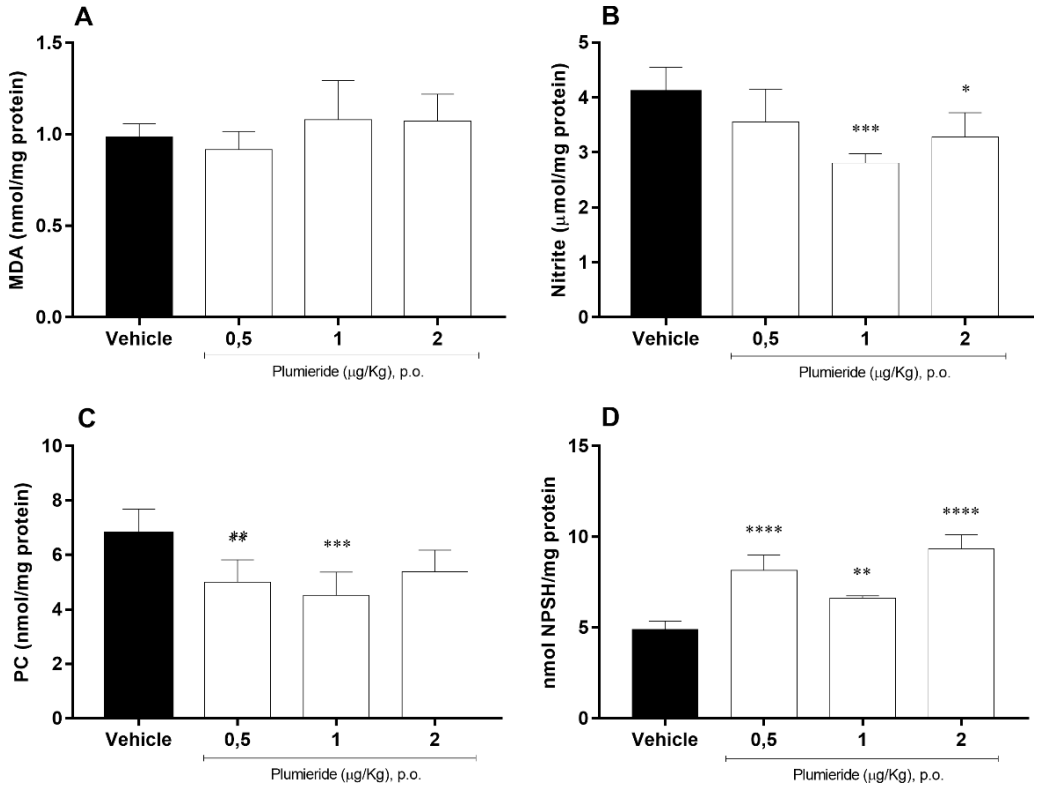


Figure 3 shows that the nitrite level was reduced in plasma of mice from PLU 1 ($p<0.001$) and PLU 2 ($p<0.05$) groups. A reduction in protein carbonylation was also observed with PLU 0.5 ($p<0.01$) and PLU 1 ($p<0.001$) treatment. In plasma, all doses of PLU increased NPSH production ($p<0.01$; $p<0.0001$). The anti-inflammatory and antinociceptive effects triggered by PLU could be related to the ability of the iridoid in performing functions in various tissues (BOEING et al., 2018; PANDYA et al., 2013; SINGH et al., 2014; SINGH et al., 2017). These effects could also be related to decreased nitrite production and protein carbonylation, which are products of inflammatory and oxidative processes (PANDYA et al., 2013; SLAVICH; IRWIN, 2014).

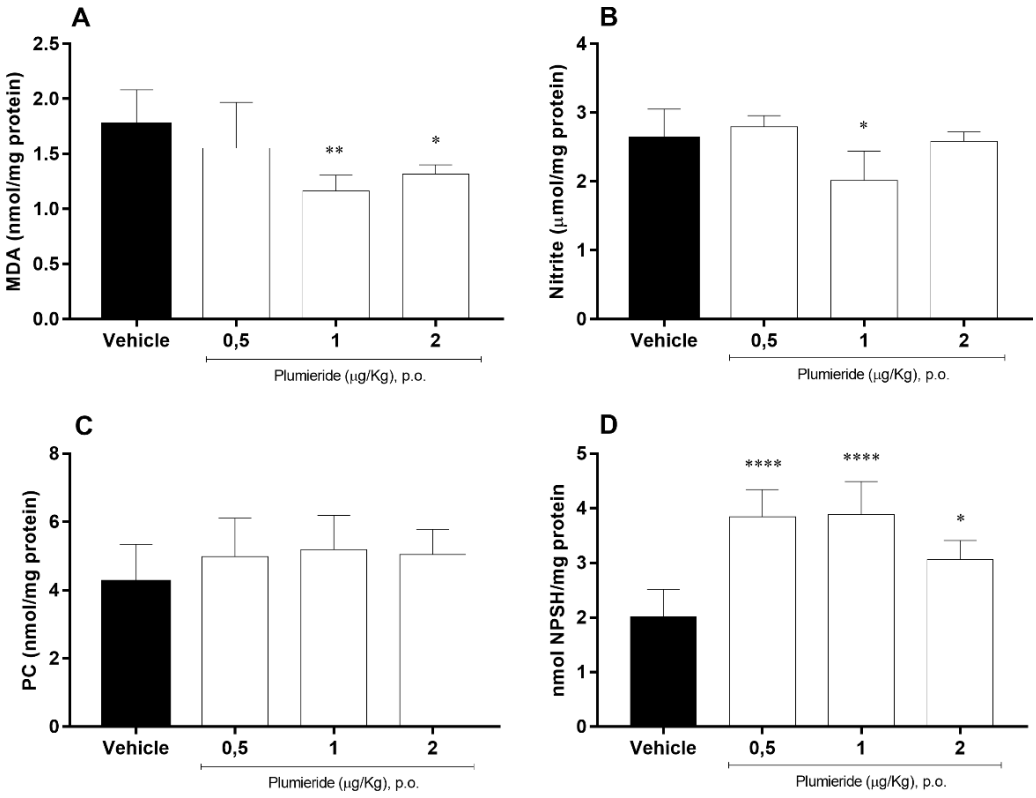
Figure 3. Effects of vehicle (distilled water, p.o.) or PLU (0.5, 1 or 2 µg/kg, p.o.) on the oxidative parameters measured in the mice´s plasma after administration for 7 days: **(A)** Lipid peroxidation (TBA-RS); **(B)** Nitrite level; **(C)** Protein carbonyl content; **(D)** Non-protein thiols groups. Results are expressed as means ± S.D. – n=5-6. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 when compared to vehicle-treated control (one-way ANOVA followed by Tukey posthoc test).



The results obtained after liver analysis (Fig. 4) demonstrate that PLU 1 and PLU 2 reduced the TBA-RS. Only mice treated with PLU 1 ($p<0.05$) showed a reduction in nitrite level. Importantly, PLU was able to raise NPSH ($p<0.0001$) in all treated-groups. Antioxidant effect of plumieride in rats' hepatic tissue was also described by Singh et al. (2017) after oral

treatment with the iridoid (5, 10, and 20 mg/kg) for 30 days, against CCl_4-induced damage. The authors also cited the normalization of other stress markers as superoxide dismutase, catalase, reduced glutathione, glutathione peroxidase, and glutathione reductase.

Figure 4. Effects of vehicle (distilled water, p.o.) or PLU (0.5, 1 or 2 µg/kg, p.o.) on the oxidative parameters measured in the mice´s liver after administration for 7 days: **(A)** Lipid peroxidation (TBA-RS); **(B)** Nitrite level; **(C)** Protein carbonyl content; **(D)** Non-protein thiols groups. Results are expressed as means ± S.D. n=5-6. *p<0.05, **p<0.01 and ****p<0.0001 when compared to vehicle-treated control (one-way ANOVA followed by Tukey posthoc test).



Finally, results from renal tissue analysis indicate that only PLU 0.5 reduced nitrite formation ($p<0.05$). However, NPSH elevation also occurred in the PLU 1 and PLU 2 groups ($p<0.05$). To the best of our knowledge, there are no mentions in the literature about the effects of PLU or another iridoid on renal tissue (Fig. 5).

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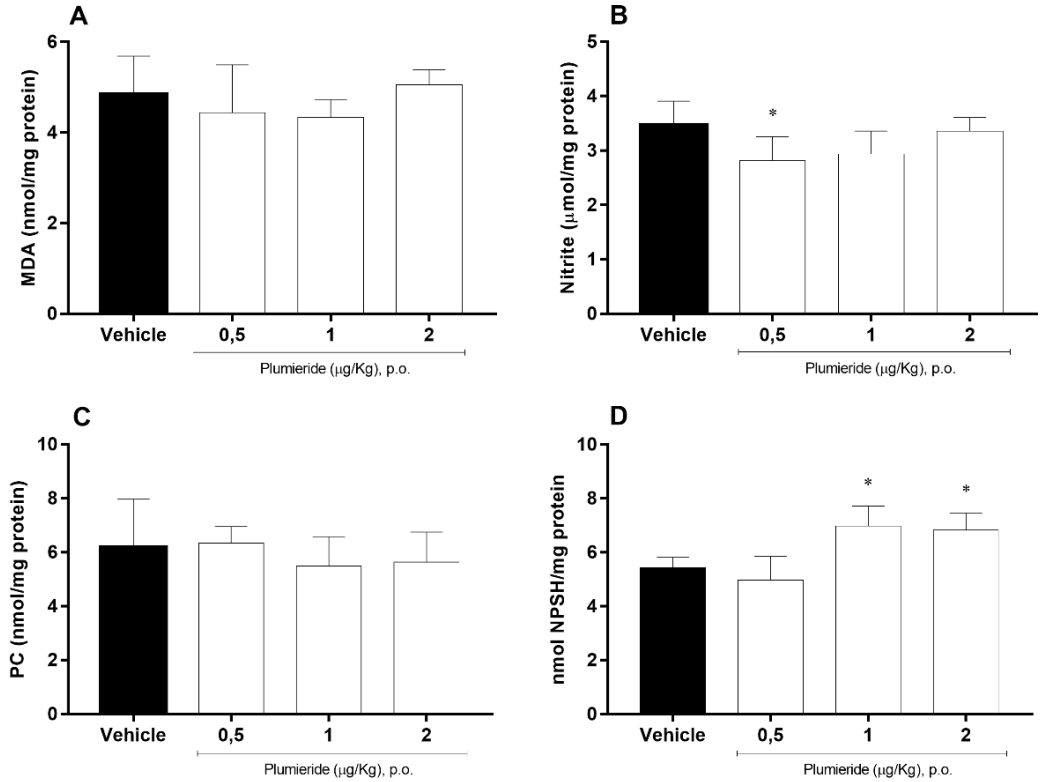
Figure 5. Effects of vehicle (distilled water, p.o.) or PLU (0.5, 1 or 2 µg/kg, p.o.) on the oxidative parameters measured in the mice´s kidney after administration for 7 days: **(A)** Lipid peroxidation (TBA-RS); **(B)** Nitrite level; **(C)** Protein carbonyl content; **(D)** Non-protein thiols groups. Results are expressed as means ± S.D. n=5-6. *p<0.05, **p<0.01 and ****p<0.0001 when compared to vehicle-treated control (one-way ANOVA followed by Tukey posthoc test).



Taken together, our results indicate that PLU has an antioxidant activity on different tissues, even after the administration of very low doses. Noteworthy, the iridoid was able to elevate non-protein thiols in all tissues. The data presented are fundamental as support for future studies, which will define the plumieride's mechanism of pharmacological action.

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Conflicts of interest: The authors declare no conflict of interest.

Use of animals: approved by CEUA 035/2016 – UNIVALI.

REFERENCES

BOEING, T. et al. Antioxidant and anti-inflammatory effect of plumieride in dextran sulfate sodium-induced colitis in mice. **Biomedicine and Pharmacotherapy**, v. 99, p. 697–703, 2018.

BONOMINI, T. J. et al. Neuropharmacological and acute toxicological evaluation of ethanolic extract of *Allamanda cathartica* L. flowers and plumieride. **Regulatory Toxicology and Pharmacology**, v. 91, 2017.

DALMAGRO, A. P.; CAMARGO, A.; ZENI, A. L. B. *Morus nigra* and its major phenolic, syringic acid, have antidepressant-like and neuroprotective effects in mice. **Metabolic Brain Disease**, v. 32, n. 6, p. 1963–1973, 2017.

DALMAGRO, A. P. et al. Avaliação de efeitos preliminares do plumierídeo após administração oral a camundongos. **Brazilian Journal of Health Review**, v. 2, n. 6, p. 5715-5730, 2019.

DONATO, F. et al. Involvement of the dopaminergic and serotonergic systems in the antidepressant-like effect caused by 4-phenyl-1-(phenylselanylmethyl)-1,2,3-triazole. **Life Sciences**, v. 93, p. 393-340, 2013.

ELLMAN, G. L. Tissue sulfhydryl groups. **Archives of Biochemistry and Biophysics**, v. 82, n. 1, p. 70–77, 1959.

GIBSON, S. A.; KORADE, Ž.; SHELTON, R. C. Oxidative stress and glutathione response in tissue cultures from persons with major depression. **Journal of Psychiatric Research**, v. 46, n. 10, p. 1326–32, 2012.

GRIESS, P. Bemerkungen zu der Abhandlung der HH. Weselsky und Benedikt „Ueber einige Azoverbindungen". **Berichte der Deutschen Chemischen Gesellschaft**, v. 12, n. 1, p. 426–428, 1879.

HUSSAIN, N. et al. New iridoids from *Lyonia ovalifolia* and their antihyperglycemic effects in mice pancreatic islets. **Fitoterapia**, v. 131, p.168–173, 2018.

LOWRY, O. H. et al. Protein measurement with the Folin phenol reagent. **The Journal of Biological Chemistry**, v. 193, n. 1, p. 265–75, 1951.

LIU, Y.-H. et al. Cytotoxic and antibacterial activities of iridoids and sesquiterpenoids from *Valeriana jatamansi*. **Fitoterapia**, v. 123, p. 73–78, 2017.

MAES, M. et al. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, v. 35, n. 3, p. 676–692, 2011.

MULLER, A.F.F. et al. Establishment of an HPLC-PDA method for analysis of derivative products from the flowers of *Allamanda cathartica*. **Journal of Chemical and Pharmaceutical Research**, v. 7, p. 250-256, 2015.

OHKAWA, H.; OHISHI, N.; YAGI, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. **Analytical Biochemistry**, v. 95, n. 2, p. 351–358, 1979.

PANDYA, C. D.; HOWELL, K. R.; PILLAI, A. Antioxidants as potential therapeutics for neuropsychiatric disorders. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, v. 46, p. 214–223, 2013.

PANKOKE, H.; BUSCHMANN, T.; MÜLLER, C. Role of plant β-glucosidases in the dual defense system of iridoid glycosides and their hydrolyzing enzymes in *Plantago lanceolata* and *Plantago major*. **Phytochemistry**, v. 94, p. 99–107, 2013.

REZNICK, A. Z.; PACKER, L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. **Methods in enzymology**, v. 233, p. 357–63, 1994.

SINGH, D. et al. Antioxidant potential of plumieride against CCl_4-induced peroxidative damage in rats. **Antioxidants**, v. 3, n. 4, p. 798–813, 2014.

SINGH, J. et al. Immunostimulatory activity of plumieride an iridoid in augmenting immune system by targeting Th-1 pathway in balb/c mice. **International Immunopharmacology**, v. 48, p. 203–210, 2017.

SLAVICH, G. M.; IRWIN, M. R. From stress to inflammation and major depressive disorder: a social transduction theory of depression. **Psychological Bulletin**, v. 140, n. 3, p. 1-80, 2014.