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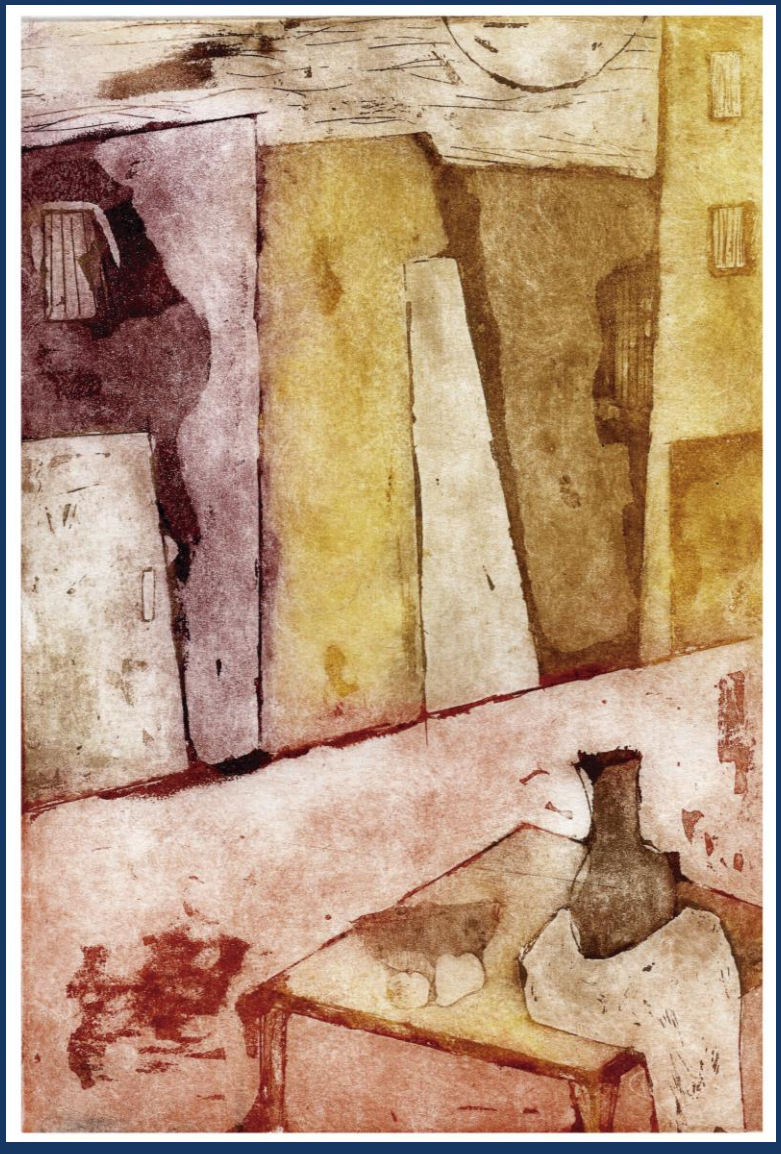

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studies, we demonstrated that autophagy participates in the elimination of intracellular amastigotes by means of xenophagy, a pathway of capture and degradation of intracellular microorganisms dependent on autophagy. Due to a direct cytotoxic action of the UA on epimastigotes, the replicative stage in the insect vector was discarded, we decided to study the action of the UA in the elimination of the amastigotes of T. cruzi in the macrophages and the possible participation of the autophagy and other mechanisms in this process. To test this, we infected macrophages with T. cruzi Y strain for 24 hours, and then treated the samples for 24, 48 or 72 hours under both control and UA (10 µM) conditions, and evaluated the amount of amastigotes by indirect immunofluorescence and western blot. We also performed cell viability tests with alamar blue to study the UA cytotoxicity on mammalian cells. Xenophagy (by IFI), and ROS generation (by 2',7'-dichlorodihydrofluorescein diacetate reaction) was also tested as two possible mechanisms of action of this drug. Our data show that UA decreases the amount of amastigotes in macrophages. We also observe that UA induces the autophagy pathway, and that LC3, the marker of autophagy, is recruited around amastigotes indicating xenophagy of these parasites. Moreover, the productions of ROS after 24 hours of treatment are increased. We conclude that UA decreases the amount of intracellular amastigotes by multiple mechanisms. UA stimulates the autophagy pathway promoting parasite capture and degradation through xenophagy. On the other hand, UA stimulates the production of ROS, which is toxic for T. cruzi but, interestingly, UA not have this effect on non-infected cells. However, we do not rule out a direct action of the UA on amastigotes, which we are studying by transmission electron microscopy.

0581 - IN VITRO ANTIVIRAL ACTIVITY OF NORDIHYDROGUAYRETIC ACID AND ITS TETRAMETHYLATED DERIVATIVE ON ARBOVIRUS WITH MEDICAL-VETERINARY IMPORTANCE

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In the search for new antiviral agents from native plant species, we began studying Larrea divaricata CAV. (Zigophyllaceae) and its main metabolite: nordihydroguaiaretic acid (NDGA). The antiviral effect of NDGA and its derivatives has been reported in numerous studies, the range of viruses evaluated is wide. However, there are few reports on arboviruses. We aimed to evaluate the in vitro effect of NDGA and its tetra methylated derivative, NDGA-4-M, on arbovirus: Chikungunya (CHIKV), St. Louis encephalitis (SLEV), Bunyamwera (BUNV) and Dengue types 1 and 4 (DENV-1, DENV-4), trying to establish the stages of the viral replication cycle affected. Cytotoxicity was assessed as a measure of the host cell viability in vitro by the neutral red uptake method. The virucidal and antiviral effects were evaluated by the plaque forming units (PFU) method. To those compounds showing active, we studied their action in several stages of the viral replication cycle, by using the UFP reduction method at different treatment times. Concentration of NDGA and NDGA-4-M causing 50 % of cytotoxicity (CC50) in LLC-MK2 cells were 115.7 µM and 7.8 µM respectively. NDGA-4-M was not able to inhibit any of the viruses tested. Although NDGA was not active also on CHIKV, SLEV and DENV-4, it was active on DENV-1 and BUNV with a selectivity index of 8.4 and 5.2 respectively. NDGA also produced an inhibition greater than 3 logarithms on DENV-1 when assessing virucidal activity. When evaluating the NDGA effect at different times of the viral replication cycle, it was determined that NDGA acts during the first two hours post-internalization (p.i.) on DENV-1 infection. By contrast, it was active all the time p.i. viral of the BUNV and, to a lesser extent, when cells were pre-treated before infection. Since there is currently no specific antiviral therapy available for the effective clinical treatment of infections produced by arboviruses, these results make NDGA a promising drug to treat these infections.

0606 - ASSESSMENT OF RESISTANCE PATTERNS OF ANTIMICROBIALS WITH HIGH IMPORTANCE IN HUMAN HEALTH, IN DIAGNOSED BOVINE NEUMONIA.

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Bovine respiratory disease (BRD) is the most important illness in feedlot cattle, where animals are subjected to high pharmacological pressure with antimicrobial (ATM) drugs for prevention or treatment. Some of ATM used for prevention or treatment of BRD, are considered by OMS as very high and high importance for human health. The aim of this study was to assess the resistance frequency of Pasteurella multocida and Mannheimia haemolytica isolated from dead cattle with BRD, in order to determine the bacterial resistance. This trial involved the analysis of 41 isolated strains from 38 dead animals. Antimicrobial susceptibility testing was performed on all isolates strains using broth microdilution and a commercially available bovine/porcine panel (Sensititre®; Trek Diagnostic Systems, Cleveland, OH, USA). The frequency of resistance for the group I of antimicrobials (agents of very high importance to human health-Fluorquinolones and 3rd generation of Cephalospirines) was a 0% for the 2 bacteria assayed. However, the frequency of resistance for Category II antimicrobials (high importance to human health—aminoglycosides, macrolides and lincosamides) was variable. Thus, the resistance pattern obtained for clindamycin was 47.6 and 55 % for either P. multocida and M. haemolytica, whilst for tilosyn was 19 and 100 %, respectively. The 21.9 % of the strains analyzed did not show resistance to any class of the antimicrobials tested. Regarding the multiresistance pattern, the 26.9 % of the strains showed resistance to only one antimicrobial group while the 51.2 % of the strains showed resistance to 2-4 antimicrobials. M. haemolytica resulted the bacteria with the highest resistance levels in animals died by BRD disease. The determination of the frequency of resistance pattern is essential to rationalize the therapeutic in animals subjected to high pharmacological pressure, in order to design new treatment interventions for reducing the development of bacterial resistance.

0614 - HIGH-THROUGHPUT MUTATIONAL ANALYSIS OF TRYPANOSOMA CRUZI ANTIGENIC EPITOPES REVEAL CONSISTENT CONSERVATION OF KEY RESIDUES ACROSS HUMAN CHAGAS DISEASE POPULATIONS.

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Chagas Disease is a major health problem for which no vaccine for public health interventions are yet available. Diagnosis is essential