Methods: The effect of silibinin and VOsil on the human lung cancer cell line (A549) viability was measured (MTT assay). In addition, the effect of the compounds at non-cytotoxic concentrations (5 μM) on adhesion, migration and invasion was investigated. On the other hand, the interaction between both compounds and BSA was investigated using tryptophan fluorescence quenching.
Results: VOsil behaved as a more cytotoxic agent than the ligand at concentration 100 μM inhibiting 40 % of cell viability. The adhesion to fibronectin ability of cells treated with silibinin and VOsil decreased 34 and 58 %, respectively in comparison with the control. The number of migrating cells decreased about 50 % after VOsil treatment. Silibinin attenuated cell migration to a lesser extent (25%). A 40% and 23% reduction on cell invasion was observed when cells were treated with VOsil and silibinin, respectively. Usually, the oxidovanadium(IV) cation was less effective in all assays. Binding constant values for the interaction of silibinin ($9.88 \pm 0.95 \times 10^6$ $L.mol^{-1}$) and VOsil ($12.58 \pm 0.76 \times 10^6$ $L.mol^{-1}$) with BSA were determined, suggesting high affinity of the compounds toward the protein. Also, n values of 1.07 ± 0.06 (silibinin) and 1.48 ± 0.07 (VOsil) were obtained indicating an interaction with one binding site of BSA.
Conclusion: This study shows that the complexation enhances the biological effects of the free flavolignan.

248. (122) **EPSTEIN BARR VIRUS RECRUITS PDL1 POSITIVE CELLS AT THE MICROENVIRONMENT IN PEDIATRIC HODGKIN LYMPHOMA.**

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Classic Hodgkin lymphoma (cHL) is a lymphoid neoplasm in which the immune microenvironment contributes to the lymphomagenesis process. Epstein Barr virus (EBV) presence also influences cHL microenvironment composition and contributes to pathogenesis. Our aim was to evaluate the PD1 / PDL1 pathway and EBV influence on this pathway in pediatric cHL. Methods: 80 pediatric patients were analyzed. EBV presence was assessed by in situ hybridization (HIS), the expression of PDL1 in the microenvironment (PDL1mic) and PDL1 in the HRS tumor cells (PDL1HRS) and PD1 in the microenvironment (PD1mic) by immunohistochemistry (IHC) expressing the results as +cells/ mm2. PDL1 genetic alterations were analyzed in a subgroup of 37 pediatric patients by FISH, following the criteria of: genetic gain (PDL1 / CEP9 <3: 1), amplification (PDL1 / CEP9 ≥3: 1) or diplody (PDL1 / CEP9=1:1). The survival was evaluated in relation to the expression of PDL1 mic, PDL1 HRS and PD1 mic. Results: No significant differences were observed in the PD1mic count or in the PDL1 HRS count between the EBV + and EBV- cases (P> 0.05; Mann Whitney test). Unexpectedly, only 38% of pediatric cHL showed PDL1 genetic alterations by FISH (8% amplification, 16% gain and 13% gain + amplification) and no differences were observed in EBV + vs EBV- cases (p> 0.05, exact test of Fisher). In the cHL EBV + cases, a significant increase in PDL1mic + cells was detected in the microenvironment (p> 0.05, Mann Whitney test). Neither PD1mic nor PDL1 expression in HRS cells or in the microenvironment was associated with survival in pediatric patients (p> 0.05, log-rank test) .Conclusions: Although our group previously described an environment of high cytotoxicity in pediatric EBV + cHL, it could be counteracted by a PDL1 + cell niche in the microenvironment, leading to unsuccessful elimination of EBV+ HRS tumor cells.

249. (123) **ANTIPROLIFERATIVE ACTIVITY OF EXTRACTS FROM LIQUID CULTURE OF EDIBLE AGARICOMYCETES (BASIDIOMYCOTA) FUNGAL STRAINS ON HUMAN PROSTATE TUMOR CELLS**

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Fungi are eukaryotic organisms with absorbotrophic heterotrophic nutrition. They present growth with four phases: lag, exponential, stationary and senescent.The exponential phase presents a primary metabolism in charge of obtaining energy through nutrients and getting biomass. In the stationary phase they synthesize secondary metabolites (SM) that allow it to degrade the substrate and thus expand. Medicinal properties have been attributed for some of these SM. Studies on tumoral cells shown apoptosis induction, arrest of the cell cycle and impaired proliferation by effect of edible strains of the Agaricomycetes class. However, most of the studies were performed with the basidium and little is known about the effect of the extracellular medium (EM), where the SM are concentrated. In this study we aimed to evaluate the effect of the EM of different fungal strains on the viability of human prostate tumor cell. PC3 cells were cultured with EM (20%; 24h) from six different strains of fungi collected at exponential or stationary phase (days 7 or 21, respectively). We found that the EM of C. comatus, M. titans and G. lucidum, from day 21 significantly decreased ($p < 0.05$) the viability of PC3 cells, evaluated by the MTS assay; whereas both EM (7 and 21) from C. cylindracea, impair viability of PC3 cells ($p < 0.05$), with a significant decrease in the number of cells ($p < 0.05$). These effects correlate with changes in cell-cycle where S phase grows at the expense of G1 phase. Nevertheless, no changes were observed in apoptosis levels, analyzed by flow cytometry with ANXAV-FITC / IP staining. These results indicate that EM of different strains of Agaricomycetes affect the viability of tumor cells, and this effect is not only dependent of the strain, but also on the growth phase.

250. (127) **THE ROLE OF AMPK-MEDIATED AUTOPHAGY IN COLON CANCER AND ITS THERAPEUTIC IMPLICATION IN PHOTODYNAMIC THERAPY WITH METHYL-AMINOLEVULINATE**

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Colorectal cancer (CRC) is the second leading cause of cancer death. The increased resistance of CRC to conventional therapies led to the need to seek new treatments such as photodynamic therapy (PDT). However, autophagy has been recently linked to the resistance of different antitumor treatments including PDT. Although autophagy is considered mainly as a cell survival mechanism, autophagy-mediated cell death has also been described evidencing the dual role of this process whose effects on therapeutic resistance are not yet fully elucidated. The increase in adenosine monophosphate (AMP) levels in cells will activate the AMP-dependent kinase (AMPK), who induce autophagy through the inhibition of the mTOR complex. Based on the above, we propose to characterize the mechanisms that govern autophagy in CRC through the AMPK-induced pathway and its implication in the therapeutic response to PDT. For this, we used the GEPIA and Human Protein Atlas database to study the relevance of AMPK and its correlation with the main modulators of autophagy in CRC. We also established two autophagy induction systems using SW480 CCR cells. For the AMPK-dependent pathway, cells were incubated in phosphate buffer saline (PBS 1X, 15min - 2 h) and for the AMPK-independent pathway, Compound C (10-20 µM, 18 and 8 h) in growth medium was implemented. It was shown that the induction of autophagy, by both conditions, produces a decrease in cell viability (MTT) in CRC cells (p value < 0.0001) over time where apoptosis is the main mechanism of cell death (determined by an Annexin V/Propidium iodide assay 24 hours post-autophagy induction measured by flow cytometry). Lastly, it was shown that cells with active autophagy (dependent and independent of AMPK) does not affect the cell viability (MTT) of CRC cells (p value = ns) treated with PDT (1.8 J/cm^2; Me-ALA 0.5 mM); which suggests that neoadjuvant treatments, which induce autophagy through these pathways, could be beneficial for the success of PDT.