10. Cancer and Cell Proliferation

10.001 **Evaluation of antitumor activity of new analogues from combretastatin A4**. Sales NM[1], Amaral DM[2], Oliveira LN[2], Lima LM[2], Barreiro EJ[2], Fernandes PD[1] [1]ICB-UFRJ, [2]LASSBio

Introduction: According to World Health Organization (WHO), in 2005 cancer was responsible for 13% of deaths worldwide. In Brazil, cancer was the fourth leading cause of death in the same year. The combretastatins are natural compounds extracted from the South African tree *Combretum cafrum* (PETTIT, GR et al. Experientia, 209, 1989) and were the first agents identified as depolymerizing of microtubules that had the ability to change the tumor vasculature (DARK GG et al. Cancer Res., 1829, 1997). The combretastatin A4 (CA-4) strongly binds to tubulin, inhibits microtubule formation and has cytotoxic activity against a variety of tumor cell lines, including multiple drug resistant cells, which makes CA-4 a potent anti-cancer drug. In this work our aim was to evaluate the cytotoxic effects of new CA-4 analogues against melanoma cell line (B16-F10). **Methods**: The synthetic CA-4 analogues were Loe-01, Loe-03 and Loe-09. Tests of cell viability through mitochondrial respiration were based on the metabolic reduction of the bromide of 3-(4,5-dimethylthiazol-2-yl)-2,5-difenltetrazolium (MTT) to formazan. The cytotoxic effect was evaluated after incubation of murine melanoma cells (B16-F10) with 1μM, 10μM or 30μM of each substance, for 24 or 48 hours. After 24h incubation, when compared to control group, Loe-01 reduced cell viability in 16% (1μM), 19% (10μM) and 20% (30μM). Loe-03 inhibited 26% (1μM), 35% (10μM) and 42% (30μM). Loe-09 had the maximal inhibitory effect with 56% (1μM), 49% (10μM) and 52% (30μM). After 48h incubation, Loe-01 reduced cell viability in 16% (1μM), 17% (10μM) and 31% (30μM). Loe-03 reduced in 12% (1μM), 18% (10μM) and 20% (30μM) and Loe-09 inhibited in 15% (1μM), 21% (10μM) and 22% (30μM). CA-4 was also tested with 1μM, 10μM or 30μM concentration and compared to control group, its inhibitory effect was 36%, 22% and 21% after 24h incubation. After 48h, CA-4 induced 57%, 36% and 42% of cell death. These data are consistent with Mouset et al. (Bioorg. & Med Chem, 3266, 2008), which showed that others derivatives of CA-4 inhibits growth of several tumor cell lines (at concentrations between 20 and 50 nM) due to its anti-mitotic action. In vivo tests will be performed using the analogues with maximal effects, following principles and norms of the Brazilian Council for Animal Experimentation (COBEA) and the committee of animal use in research of the Center for Health Sciences (CAUAP)/UFRJ, under the authorization DFBCICB-015. The search for new synthetic molecules with significant antitumor activity and decreased risk of incidence of side effects should always be the focus of studies. Thus, the possibility of finding new substances can bring long term benefits if they become new drug candidates. **Conclusions:** Combretastatin A-4 is known as potent cytotoxicity inductor and at inhibiting microtubule polymerization and cell metastasis. These findings suggest that it is possible to make analogues that can lead to a better result in clinical trials. Financial Support: CAPES, FAPERJ, INCT-INOFAR, IVB.

10.002 **Irradiation modulates IL-17R expression on human glioma cell line**. Gehring MP[1], Pereira TCB[2], Borges MC[3], Braga Filho A[3], Bogo MR[2], Campos MM[4], Morrone FB[1,4] [1]PUCRS – Farmacologia Aplicada, [2]PUCRS – Genômica e Biologia Molecular, [3]HSL – Radioterapia, [4]PUCRS – Toxicologia e Farmacologia

Introduction: Glioma is the most common and lethal type of primary brain tumor (Karrlander et al., *PLoS One* 4:e8536 – 2009) and has disproportionately high mortality rate of more than 70% of cases in two years after diagnosis (Filippi et al., *PLoS One* 6:e20849 – 2011). Radiotherapy is a mainstay therapy for patients with glioma (Weller et al., *Swiss Med Wkly* 141:w13210 – 2011). However, it remains only a palliative therapy because glioma cells display significant radioresistance (Van Meir et al., *CA Can J Clin* 60(3): 166 – 2010). Inflammatory cells and molecules may have crucial role in tumor microenvironment (Zou et al., *Nat Rev Immunol* 10:248 – 2010). Interleukin-17 is a pro-inflammatory cytokine secreted predominantly by activated T-cells (Ivanov et al., *Trends Pharmacol Sci* 30:95 -2009). Recently, a study has confirmed the presence of the IL-17A in both human and mouse glioma cells (Wainwright et al., *PLoS One* 5:e15390 – 2010). The aim of this study was to describe the action of IL17 on glioma cell lines and its modulation by irradiation. **Methods:** Human glioma cell (U-138) was treated with IL-17 (5, 10 and 20 ng/ml) and cell viability was assessed by MTT assay. For the irradiation study, U-138 and M059J human glioma cells were irradiated (2 Gy), after 24 h total RNA was isolated with Trizol. The IL-17R expression was accesses by qPCR-RT. Data were analyzed by ANOVA, followed by Tukey–Kramer post test using GraphPad Software (San Diego, CA, U.S.A.). p values <0.05 were taken to indicate statistical significance. **Results:** The cell viability was decreased when the human glioma cell U-138 was treated with IL-17 5, 10 or 20 ng/ml (53.7% ± 5.70, 66.3% ± 1.22 and 55.5% ± 0.70, respectively). Treatment with irradiation (2 Gy) caused a marked increase in IL-17R expression on both lineages (U-138 and M059J) when compared to their respective controls (37.5%, from 0.40 ± 0.03 to 0.55 ± 0.02 and 31.5%, from 0.54 ± 0.03 to 0.71 ± 0.05), proposing that IL-17R could be related with irradiation response. The expression of IL-17R on M059J radiosensitive line was significantly higher when compared to U-138 radioresistance cell line (0.54 ± 0.03, and 0.40 ± 0.03, respectively). Moreover, similar results of IL-17R expression were obtained when the lineages where studied 24 h after irradiation (2 Gy) (0.71 ± 0.05 and 0.55 ± 0.02, respectively). **Discussion:** Our results show that the presence of IL-17 in the cell culture medium decreases the glioma cell viability. Furthermore, this study provides the first report about the modulation of IL-17R by radiotherapy, indicating that this cytokine can be related to irradiation response. Accordingly, we propose that the increase of IL-17R on the human glioma cells could stimulate a pro-inflammatory tumor microenvironment. **Financial Agencies:** CAPES, FAPERGS, FINEP and PUCRS.

10.003 **Anticancer activity of fraction containing diterpenes from *Croton campestris A.St.-Hil*.**
Monteiro PA[1], Longato GB[1], Cabral E[2], Tinti SV[1], Ruiz ALTG[1], Eberlin MN[2], Foglio MA[1], Carvalho JE[1]
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Introduction: Factors such as the rise in life expectancy and bad life habits are increasing the incidence of cancer because of exposition to carcinogens. Cancer is the second leading cause of death worldwide. Of the chemotherapeutics used in cancer treatment, approximately 60%, comes from natural sources like plants (Cragg & Newman, *J Nat Prod*, 5(3):311, 2012). *Crotoncampestris* A.St.-Hil. (Euphorbiaceae) has been used by folk medicine as anti-inflammatory, antihelminthic, antimicrobial, molluscide and tumor treatment (Babili *et al.*, *Phytotherapy*,77:384, 2006). We evaluated the anticancer activity of fraction CFqb14 obtained from *C. campestris* and investigated its active principles involved with the pharmacological activity. **Methods:** *C. campestris* were collected at CPQBA's experimental field. Dried and crushed leaves were extracted with ethyl acetate providing the crude extract. This extract was further fractionated by column chromatographic with a 1:3 ratio of sample:stacionary phase. Gradients of hexane/dichloromethane/methanol of increasing polarity were used as mobile phase. The resulting fractions were evaluated. The antiproliferative activity of the resulting fractions was evaluated in the human cancer cell lines U251 (glioma), MCF7 (breast), NCI/ADR-RES (resistant ovary), 786–0 (kidney), NCI-H460 (lung), OVCAR-3 (ovary), HT29 (colon) and K562 (leukemia) and a normal cell line [HaCat (human keratinocyte)]. After 48h of treatment, cell proliferation was determined by spectrophotometric measurements in triplicate using sulforhodamine B assay. Fraction CFqb14 was evaluated in solid cancer paw model through the inoculation of Ehrlich tumor cells ($2{,}5x10^6$cell/60µL) in the right hind paw of balb/C female mice. The tumor growth was evaluated by the difference between measured volume and basal volume every three days, until 15 days after cell inoculation. Five groups (8 animals/ group) were treated intraperitoneally with vehicle (NaCl 0.9%), doxorubicin 3mg/kg (positive control) and CFqb14 1, 2 and 4 mg/kg, every three days, until 15 days after cell inoculation (Committee for Ethics in Animal Research at UNICAMP, protocol 2280-1). Fraction´s chemical composition was evaluated by HRESI-MS using a Q-Tof Mass Spectrometer (Micromass – U.K.), direct inset, in both positive and negative ion modes. **Results and Discussion:** CFqb14 showed anti-proliferative activity with selectivity mainly for NCI-H460 (lung, GI_{50} < 0,25 µg/mL). After 15 day treatment, tumors in groups treated with all doses of CFqb14, as well positive control group, were significantly lower than the vehicle group. Tumor volume was reduced by 22,5% for CFqb14 higher dose. MS analysis identified two diterpenes in CFqb14, velamone and velamolone, which could be related to anticancer activity observed for this fraction. These data demonstrated CFqb14, obtained from *Croton campestris* leaves, has a promising anticancer activity with high potency despite not yet purified. Studies on active principles isolation and mechanism of action are in progress. **Acknowledgments:** We thank biologist Benício Pereira for help in collecting of the plant material. **Support:** CNPq, CAPES, FAPESP, FAEPEX.

10.004 **Antitumoral effect of *Bothrops* venoms on cells of nervous system human (SF-295)**. Morais ICO[1], Jorge RJB[2], Martins AMC[3], Ximenes RM[2], Martins AMA[2], Rodrigues FAR[2], Soares BM[2], Evangelista JSAM[4], Toyama MH[5], Moraes MO[2], Monteiro HSA[2] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Physiology and Pharmacology, [3]UFC – Clinical and Toxicological Analysis, [4]UECE – Veterinary, [5]Unesp-CLP – Chemistry of Macromolecules

Introduction: Snake venoms represent an essentially unexplored source of bioactive compounds that may cure disease conditions which do not respond to currently available therapies. These venoms possess many pharmacological activities, as cytotoxic and/or lytic effects on tumor cells in vitro. The aim of this study was to investigate the cytotoxicity in vitro of *Bothrops pirajai, Bothrops diporus* and *Bothrops pauloensis* venoms in tumor cell lines SF-295 (nervous system - human). **Methods**: Cytotoxicity was available by assay MTT. To elucidate the mechanisms involved in the cytotoxic action of *Bothrops* venoms (10 and 20 µg/mL) in SF-295 cells, treated cells were double stained with annexin V and PI, and then analyzed by flow cytometry after 24 h of incubation with the tested venoms. The study protocol was approved by Ethics Committees from the Federal University of Ceara, in Fortaleza Brazil (n° 107/07). **Results and Discussion:** *Botrhops* venoms showed significant cytotoxicity against cell line in study. The results of the analysis of cell membrane integrity revealed the presence of a necrotic population on the 20 µg/mL concentration of *B. pauloensis* and *B. diporus* venoms, and at both tested concentration of *B. pirajai* venom. At both tested concentrations, all the venoms led to the exposure of phosphatidylserine which pointed to apoptotic cells. In conclusion *B. pauloensis, B. diporus* and *B. pirajai* venoms induce cytotoxicity activity against tumor cell line SF-295, including necrosis and apoptosis, suggesting that the venoms contain some substance(s) of therapeutic potential. Further research is needed to determine their isolated compounds may be involved in this toxic effect. Financial support from CNPq and FUNCAP.

10.005 **Antiproliferative activity of lactones obtained by synthesis on tumor cell lines**. Silva PBN[1], Freitas JCR[2], Oliveira RA[2], Menezes PH[2], Silva TG[3], Andrade JKF[3], Militão GCG[4] [1]UFPE – Fisiologia e Farmacologia, [2]UFPE – Química Fundamental, [3]UFPE – Antibióticos, [4]UFPE – Fisiologia e Farmacologia

Introduction: (-)-Massoialactone, an α,β-unsaturated δ-lactone, first isolated from *Cryptocarya massoia*, and five analogs were synthesized. Their antiproliferative activity was evaluated on tumor cells lines. **Methods**: Firstly, the antiproliferative effect of 6 compounds, (-)-Massoialactone, 1 and α,β-unsaturated δ-lactones 2a-e, were evaluated by MTT on HL-60 (pro-myelocytic leukemia), K562 (chronic myelogenous leukemia), HT29 (colon carcinoma), NCI H292 (lung carcinoma) and MCF-7 (breast carcinoma) human cell lines after 72h of treatment. Then, the effect of the most active compound on HL60 cell viability and on cell morphological alteration was determined after 24 h by tripan blue exclusion test and giensa-may-grunwald staining, respectively. **Results and Discussion**: (-)-Massoilactone, 1 displayed moderate cytotoxic activity against all tested cancer cell lines, being more active against leukemia HL-60 cancer cells. Among the tested compounds, analog 2b displayed better cytotoxic activities, with IC_{50} values ranging from 0.17 to 4.6 μg/mL. The other compounds showed the following cytotoxic potency order: 2c>2d. IC_{50} values for compound 2a and 2e were higher than 25μg/mL. Tripan blue exclusion test showed a reduction on HL60 viable cell number after 24 h treatment with 2b at 1μg/mL (41% inhibition) and at 2μg/mL (69% inhibition). Morphological analysis of the cells treated with 2b at a concentration of 2μg/mL, indicated features of apoptotic cells (reduction of cell volume, picnotic nucleus and chromatolysis). Conclusion: Among all derivatives, compound 2b was the most active indicating its potential as an anticancer agent. **Financial Support**: CNPq, UFPE.

10.006 ***Myracrodruon urundeuva*: A citotoxicity study**. LIMA DJB[1], Ferreira PMP[2], Farias DF[3], Viana MP[3], Souza TM[3], Vasconcelos IM[4], Soares BM[1], Pessoa CO[1], Moraes MO[1], Carvalho AFU[3] [1]UFC – Fisiologia e Farmacologia, [2]UFPI – Ciências Biológicas, [3]UFC – Biologia, [4]UFC – Bioquímica e Biologia Molecular

Introduction: Brazil possesses the largest diversity of plant species in the world, but less than 10% have been evaluated with respect to their biological characteristics, and fewer than 5% have been subjected to detailed phytochemical studies. In Northeastern Brazil, a region with approximately 1,539,000 km2, with warm and dry climate, grows the peculiar xerophitic "Caatinga" vegetation (dry land vegetation). In *Caatinga* flora, there are almost 1000 vascular plant species and due to the extreme climate conditions most species are endemic Despite this great biodiversity, Northeastern Brazilian plants are relatively underexploited with regard to discoveries of active biological substances. The present study aimed to assess the antiproliferative and cytotoxic potential against tumor lines of ethanolic seed extracts of 21 plant species belonging to different families from Northeastern Brazil. In addition, some underlying mechanisms involved in this cytotoxicity were also investigated. **Methodology** Firstly, the cytotoxicity of the extracts was evaluated by MTT assay on leukemia (HL-60), colon (HCT-8), melanoma (MDA/MB-435) and glioblastoma (SF-295) cancer lines after 72h exposure (0.019-25 µg/mL). HL-60-treated cells were evaluated for cellular membrane integrity, internucleosomal DNA fragmentation and mitochondrial depolarization by flow cytometry (Guava EasyCyte Mine) after 24h exposure (6.25, 12.5 and 25 µg/mL). To morphological evaluation, cells were stained with Hematoxylin-Eosin and examined by light microscopy Finally, *in vitro* antiproliferative activity on Sarcoma 180 cells was performed by Alamar Blue assay. Doxorubicin (0.3 µg/mL) was used as positive control. **Results and discussion** Only the ethanolic extract obtained from*Myracrodruon urundeuva* seeds (EEMUS) showed *in vitro* cytotoxic activity against human cancer cells, being 2-fold more active on leukemia HL-60 line [IC_{50} value of 12.5 (9.5-16.7) µg/mL] than on glioblastoma SF-295 [IC_{50} of 25.1 (17.3-36.3) µg/mL] and Sarcoma 180 cells [IC_{50} of 38.1 (33.5-43.4) µg/mL]. Flow cytometric and morphological analyses of HL-60-treated cells displayed chromatin condensation, decrease in cell number, volume and viability as well as increasing in internucleosomal DNA fragmentation in a dose-dependent way, indicating that EEMUS activates apoptotic pathways of cell death. **Financial agencies and acknowledgements:** CNPq, Funcap, UFC, CAPES, FAPEPI.

10.007 Derivative A398 induces apoptosis and modulates the activity of PGP in chronic myeloid leukemia cell lines. Silveira AL[1], Faheina-Martins GV[1], Dantas BB[1], Vasconcelos FC[2], Costas F[2], Maia RC[2], Araújo DAM[1] [1]UFPB – Farmacologia Celular e Molecular, [2]INCa – Hematologia Celular e Molecular

Introduction: Resistance to chemotherapy agents is the main reason for treatment failure in patients with cancer, and multidrug resistance (MDR) occurs in many types of tumors. The main mechanism that gives rise to the MDR phenotype is the overexpression of drug efflux transporters such as the P glycoprotein (Pgp) in the plasma membrane. Apoptosis and anti-apoptosis pathways are also deeply related to drug sensitivity and resistance. Therefore, the development of compounds that modulate the activity of Pgp and induce apoptosis is one of the therapeutic strategies in the field of oncology. In this context, a novel derivative of podophyllotoxin, a natural cyclolignan with potent antitumor activity, was synthesized and its potential anticancer was characterized in chronic myeloid leukemia cell lines. **Methods**: The cytotoxic effect of a novel podophyllotoxin derivative, called A398, was evaluated in K562 and K562-Lucena (a cell line overexpressing P-glycoprotein) lineages. Cells were treated with the derivative A398 (2 - 50 μM) for 24, 48 and 72h. Etoposide (1 - 100 μM) was used as a positive control. The cell viability was analyzed by the MTT assay. To assess the type of cell death induced, cells were incubated with the derivative A398 (4, 6, 8 and 10 μM) for 48 hours. Flow cytometry was performed for detection of phosphatidilserine exposure. The expression of anti-apoptosis proteins survivin and XIAP in leukemia cell lines, treated whit 8 μM derivative A398 for 24, 48 and 72 hours, was evaluated by western blot. For Pgp activity, K562-Lucena cells were incubated with the derivative A398 (4 - 25 μM) and functional evaluation of rhodamine 123 efflux was carried out by flow cytometry. **Results and Discussion**: The derivative A398 reduce cell viability in a time and concentration dependent manner. The IC_{50} values of K562 and K562-Lucena cells after A398 treatment for 72 hours were 8.1 ± 2.0 μM and 8.6 ± 1.8 μM, respectively. Etoposide was not cytotoxic to K562-Lucena cell line. Flow cytometry analyses showed that A398 induced apoptosis in a concentration-dependent manner. When the cells K562 and K562-Lucena were treated with 8 μM of derivative A398, the percentage of apoptotic cells was 83.0 ± 2.85% and 75.2 ± 2.46%, respectively. In these cell lines, apoptosis induced by derivative A398 was accompanied by a reduction of anti-apoptotic proteins XIAP e survivin expression. In K562-Lucena cell line, this inhibition being significant only at 72 hours. The derivative A398 decrease efflux activity of Pgp at all concentrations, suggesting that induction of apoptosis in K562-Lucena cells may be related to the inhibition of Pgp. These results show that the derivative A398 has an anticancer potential. **Financial support**: CAPES, CNPq

10.008 **Protective effect of exercise-induced oxidative stress against 1.2-dimethylhydrazine colon cancer in C57BL/6 mice**. Ribeiro RF[1], Alves GA[1], Aboulafia J[1], Rosa EF[2,1], Nouailhetas VLA[1] – [1]Unifesp – Biofísica, [2]UCS

Introduction: intense and poorly designed exercise causes damage in various tissues which are related to increased production of free radicals. Published data suggest a strong relationship between oxidative stress and carcinogenesis. In contrast a recovery of colon cancer is described in individuals who practice intense physical activity. **Objective:** compare markers of oxidative stress induced either by intense and exhaustive or moderate exercise which would lead to retard the progression of colon cancer induced by 1.2-dimethylhydrazine (1.2-DMH) in C57BL/6 mice. **Methods:** animals were divided in 6 groups: sedentary (CT-), subjected to intense and exhaustive exercise (IEE-), moderate exercise (MOD-), sedentary treated with 1.2-DMH (CT+), subjected to intense and exhaustive exercise treated with 1.2-DMH (IEE+), and moderate exercise treated with 1.2-DMH (MOD+). Induction of cancer: animals were weekly treated with a subcutaneous injection of 1.2-DMH for 6 weeks from their second week of life. When the animals completed 3 months age they were submitted to the following exercise protocol: five days of adaptation, maximal incremental test I, 10 days of a daily session treadmill running at either 85% Vmax until exhaustion (EII) or 30 minutes at 60% Vmax (MOD), and maximal incremental test II. Oxidation of total proteins of the colon was evaluated by the level of carbonyl radicals. **Results:** in the first week of treatment with 1.2-DMH CT- and CT+ animals had a similar weight. In the following weeks of treatment there was a linear increase in the weight of both animal groups such that at the end of the sixth and last week of cancer induction, the weights were statistically equal. The treatment with 1.2-DMH did not alter the animal's weights. As expected oxidation of total proteins in CT+ group increased 6x as compared to CT- (CT- = 6.013 ± 0.344 nmol/mg of protein and CT + = 40.079 ± 0.804 nmol/mg of protein). Corroborating our hypothesis there was no difference among the CT- group and IEE+, MOD+, and MOD- groups, but an increase of about 3x in the EEI- group relative to CT- group. **Discussion:** It is known that 1.2-DMH treated animals do not have their weight changed during the treatment; these data are consistent with that found in the present study. As no difference were found in physical performance between the treated animals and the non treated ones, we compared the IEE+ and MOD+ groups with the IEE- and MOD- groups. The exercise dramatically reduced the 1,2-DMH-induced oxidative stress, thus favoring a strong relationship between oxidative stress and colon cancer, although the confirmation of colon cancer needs to be evidenced by histological studies. **Financial Support:** FAPESP; 2010/13317-4 **CEP/UNIFESP:** 1774/10

10.009 **Evaluation cytotoxic of derivative hydantoinic in heLa**, **PC3 And CHO cells**. Aguiar ACV[1], Câmara RBG[1], Rocha HAO[1], Lima MCA[2], Galdino SL[2], Pitta IR[2], Carvalho MS[3] [1]UFRN – Bioquímica, [2]UFPE – Antibióticos, [3]UFRN – Biofísica e Farmacologia

Introduction: Several synthetic molecules can be obtained from derivatives heterocyclic rings, among which stand out imidazolidines-2,4-diones (hydantoins), due to its potential as a prototype for the development of new drugs. The molecular modification of imidazolidines by radicals replacement produces different biological responses [1] such as antimicrobial, anticonvulsant, schistosomicidal [2], analgesic [3] and anti-inflammatory [4]. Although hydantoin compounds are studied extensively, there are not many studies that investigate their anticancer properties. **Methods:** The compound **LPSF/MS-02** [3-(4-methyl-benzyl)-5-(4-chloro-benzylidene)-imidazolidine-2,4-dione] was synthesized and tested on the cancer cell lines: HeLa (cervical carcinoma – human), PC3 (prostate carcinoma – human) and CHO (tumorigenic ovarian cells – chinese hamster). For comparative purposes we used an anticancer drug used in the clinic, doxorubicin (Dox), as positive control. The citotoxicity was evaluated by the MTT test. The compounds previously dissolved in DMSO were diluted in DMEM or RPMI to obtain the final concentrations (5,10,25 and 50 μM) and added in 96-well microplates (100μL/well), with a cellular density of $5x10^3$ cells/well. The cells were incubated in the presence of LPSF/MS-02 by 24,48 and 72h at 37°C and 5% CO_2, in triplicate. After each period, the supernatant was aspirated, was added 100μL of MTT (5 mg/ml in DMEM, except for PC3 where was used RPMI) and the microplates were replaced in an incubator for 4h. After that, the supernatant was aspirated and the formazan product was solubilized by the addiction of 100μL of ethanol. The plates were agitated during 15 minutes and the absorbance was read on spectrophotometer at a wavelength of 570nm [5]. Statistical analysis was performed according to their means and their standard errors and deviations were made from non-linear regression using GraphPad Prism (version 5). **Results and Discussions:** The LPSF/MS-02 at a concentration of 5 μM and at the first 24h showed 58,8% of cellular proliferation in comparison with 82,4% of the antineoplasic drug Dox, in the PC3 cell line. For the CHO the compound showed a close effect to the Dox, and for the HeLa it was not as effective. The different cytotoxic effect exhibited by the LPSF/MS-02 occurred probably because the constitutive and peculiar characteristics of each cellular type and/or due to different pathways of action on the metabolism of these cells. **Conclusions**: The derivative of hydantoin was more selective for the prostate carcinoma cell line, and proved to be more effective than the doxorubicin and promising anticancer drug, requiring further investigations on its cytotoxic mechanism. References: 1. Pitta M. G. R. Mem. Inst. Oswaldo Cruz 101: 313. 2006. 2. Oliveira S. M. Quim.Nova 31:614. 2008. 3. Sudo R. T. J. Pain 11:71. 2010. 4. Guerra A.S.H.S. Int. Immunopharmacol. 11:1816. 2011. 5. Mosmann T. J. Immunol. Methods 65:55. 1983. Financial Agencies and acknowledgments: BIOPOL/UFRN Laboratório de Farmacologia/UFRN Capes

10.010 **Targeting the stress response as a selective mechanism to kill cancer cells**. Marinho-Filho JDB[1], Araújo AJ[1], Pessoa C[1], Costa MP[1], Diniz JC[2], Viana FA[2], Pessoa OLP[3], Silveira ER[3], Moraes MO[3], Costa-Lotufo LV[1] [1]UFC – Fisiologia e Farmacologia, [2]UERN – Química, [3]UFC – Química Orgânica e Inorgânica

Introduction: Natural products are the key molecules in the development of anticancer drugs, and many studies have been conducted aiming the identification of promising compounds among Brazilian biodiversity. Cordiaquinones are meroterpenoids from plants belonging to the genus *Cordia* with several described biological activities, including antifungal, larvicidal and cytotoxic effects. **Methods**: The cytotoxicity of (+)-Cordiaquinone J (purity above 98%), isolated from the roots of *C. leucocephala* Moric*,* was tested against thirteen human cancer cell lines and two normal cells lines by MTT assay with some inhibitors of apoptosis and oxidative stress pathway. To further investigate the mechanisms involved in the cytotoxic activity, the effect of (+)-Cordiaquinone J on DNA fragmentation, cell cycle, mitochondrial depolarization, caspases activation and ROS generation were performed by flow cytometry in HL-60 cell line, using doxorubicin and/or β-lapachone as positive controls. C-myc, DNA damage and apoptosis related proteins were detected by western blot. The *in vivo* antitumor activity was confirmed in mice bearing sarcoma 180 tumor orally treated with (+)-Cordiaquinone J (25 or 50 mg/kg/day during 7 days). All experimental procedures were approved (protocol #35/10) by the Institutional Animal Ethics Committee (CEPA/UFC). **Results and Discussion:** The compound showed IC_{50} values ranging from 4.6 to 6.8μM in leukemia cells and 33.6 to 37μM in normal cells. No significant difference was observed in IC_{50} values when the cells were treated with BAPTA (an intracellular Ca^{2+} chelator), dicoumarol (inhibitor of NAD(P)H quinone oxidoreductase-1 (*NQO1*) or cyclosporin A (mitochondrial permeability transition pore inhibitor), however when the cells were treated with inhibitors of reactive oxygen species (ROS) (N-acetyl-L-cysteine, (NAC) or ascorbic acid) was observed a large decrease in activity of the compound, but this activity was not changed when the cells were treated with tocopherol or alpha-lipoic acid. The DNA fragmentation after 3 hours of incubation was inhibited after the use of specific inhibitors of caspases 3/7 and 8, but was not inhibited after the use of inhibitors of caspase 9. The effects of mitochondrial depolarization and loss of cell membrane integrity was not altered when incubated with inhibitors of caspases. (+)-Cordiaquinone J altered the redox potential of cells by inducing the depletion of reduced GSH intracellular content, the generation of ROS and the loss of mitochondrial membrane potential. (+)-Cordiaquinone J induced both necrosis and apoptosis and decreased of C-Myc levels in HL-60 cells. (+)-Cordiaquinone J also promoted DNA damage activating protein kinases of the ATM/ATR pathway in tumor cells but not in normal cells in a mechanism dependent of the generation of ROS. The anticancer potential was confirmed *in vivo* through inhibition of Sarcoma 180 tumor by 72.5% at 50 mg/kg. The pre-treatment of cells or animals with NAC abolished most of the *in vitro* and *in vivo* observed effects, reinforcing the role of ROS generation in (+)-Cordiaquinone J activity. **Supported by**: CNPq, CAPES, FUNCAP and PRONEX.

10.011 **Cytotoxic effect of an abietane diterpene isolated from *Hyptis carvalhoi* (Lamiaceae) promotes cell cycle arrest in G_0/G_1 phase**. Araújo AJ[1], Lima KSB[2], Marinho-Filho JDB[1], Silveira ER[2], Moraes MO[1], Pessoa C[1], Costa-Lotufo LV[1] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Química Orgânica e Inorgânica

Introduction: HCRH is an abietane diterpene quinone isolated from the roots of *Hyptis carvalhoi* Harley. Abietane diterpene quinones are often reported to have cytotoxic effects on cancer cell lines, although their exact mechanisms of action are still unclear. The aim of this work was to evaluate the cytotoxic effects of HCRH in cancer cell lines. **Methodology:** The compound was tested against several cancer cell lines using MTT assay, after 72 hours of incubation. Cell growth was quantified by the ability of living cells to reduce MTT to a blue formazan product. To perform the hemolytic assay, a 2% mouse erythrocyte suspension was used. After incubation for 1h with compound, the supernatant containing hemoglobin was measured at 540 nm. To further understand the mechanism underlying the cytotoxicity of HCRH, studies involving membrane integrity, DNA fragmentation and cell cycle analysis were performed by flow cytometry in the human adenocarcinoma HCT-116 cell line, using doxorubicin as a positive control after 24h, 48h and 72h of incubation. **Results and Discussion:** The compound was cytotoxic against all tested cell lines, showing IC_{50} values ranging from 4.6 up to 31.6 µM in HCT-116 and HL-60 cells, respectively, after 72h of incubation. No hemolytic effects were observed (EC_{50}> 1mM). The propidium iodide intercalation test showed that HCRH caused disruption of cell membrane in HCT-116 after 48h of incubation at the highest concentrations (6.0 and 12µM). Additionally, this compound decreased the cell densities at all times and concentrations tested and caused DNA fragmentation in a dose-and time-dependent manner. Regarding cell cycle, this diterpene also influenced cell cycle progression and arrested cells in G_0/G_1 phase of the cell cycle. The results presented in this work suggest that the diterpene HCRH acts by inducing arrest in the G_0/G_1 phase of the cell cycle leading to death of the treated cells, probably, by apoptosis. Further studies are in progress to determine its mechanism of action. **Supported by:** CNPq, PRONEX, CAPES and FUNCAP.

10.012 Modulation of endothelial cells by human tumor microenvironment: **A role for synthetic analogues of lipoxins**. Vieira AM[1], Helal Neto E[1], Figueiredo CC[1], Barja-Fidalgo TC[1], Fierro IM[2], Morandi V[1] [1]DBCEL-UERJ, [2]DFP-UERJ

Tumor arises from a pathological disorder characterized by a cell growth and excessive cell proliferation. Melanoma originates from melanocytes. Angiogenesis is necessary for tumor growth and metastasis. Lipoxins (LX) and 15-epi-LX are lipids with a potent inhibitory effect on angiogenesis. Our group demonstrated that ATL-1, a synthetic analogue of 15-epi-LXA4, inhibits many actions stimulated by angiogenic vascular endothelial growth factor (VEGF). However, the actions of LX on endothelial cells in a tumor microenvironment are still unknown. Here, we investigated a modulation of human umbilical vein endothelial cell (HUVEC) by ATL-1 in a tumor's microenvironment. ATL-1 inhibited VEGF-induced permeability effect. In trials of transmigration, HUVEC were pre-treated with ATL-1 for 30 minutes and, then, stimulated with VEGF for 1 hour before the addition of MV3 cells. We observed that ATL-1 inhibited transmigration of melanoma through the endothelial monolayer stimulated by VEGF. By immunocytochemistry, we observed that this inhibition was due to the permanence of VE-cadherin on EC membrane. By Western blotting assay, we observed a reduction in the nuclear translocation of beta-catenin. By ELISA, we showed that MV3 secreted VEGF while NGM did not. ATL-1, per se, didn't reduce the secretion of VEGF by tumor cells, however, reduces its shares on the endothelial monolayer at the time of 18 hours. In conclusion, ATL-1 inhibited VEGF-induced permeability, reducing endothelial transmigration of MV3. It could be used as a modulator of tight junctions in endothelial cells, reducing tumor spread. Ethical committee - CAAE 0086.0.314.325-10. Capes, FAPERJ, CNPq, SR2

10.013 **Screening of metal complexes of ruthenium for cytotoxicity in cancer cell lines**. Soares TEL[1], Araújo AJ[1], Marinho Filho JDB[1], Sá DS[2], Fernandes FA[2], Pessoa C[1], Costa Lotufo VL[1], Lopes FGL[2], Sousa SHE[2], Moraes MO[1] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Química Orgânica e Inorgânica

Introduction: Ruthenium-based compounds represent the way for introducing a new class of antitumor drugs endowed with a great potential for the management of human tumors. The aim of this study was to analyze the cytotoxic activity of sixteen compounds for their antiproliferative activity in cancer and normal cell lines. **Methodology:** The sixteen compounds (25µg/mL) were tested against four human cancer cell lines: OVCAR-8 (ovary), HCT-116 (colon), SF-295 (glioblastoma), HL-60 (leukaemia) and peripheral blood mononuclear cells from healthy humans (PBMC) by the MTT assay after 72 h incubation. Cell growth was quantified by the ability of living cells to reduce MTT to a blue formazan product. **Results and Discussion:** From these sixteen compounds named 1 to 16, only four of them have displayed moderate cytotoxic activity (compounds 2, 7, 9 and 13). Compound 9 was the most active showing IC_{50} values of 3.72 µM in HL-60, 6.11 µM in HCT-116, 8.65 µM in SF-295 and 16.98 µM in OVCAR-8. The cytotoxicity of the compounds (2, 7, 9 and 13) was not observed in normal cells. These findings point to the potential of these ruthenium-based compounds as model molecules to produce new compounds with anticancer properties.

10.014 **Hellebrigenin-induced cell cycle arrest and apoptosis on HL-60 leukemia cells**. Soares BM[1], Cavalcanti BC[1], Rodrigues FAR[1], Cunha-Filho GA[2], Santos ML[2], Moraes MO[1], Pessoa C[1] [1]UFC – Fisiologia e Farmacologia, [2]UnB

Introduction: Hellebrigenin and other bufodienolides are cardioactive steroids of 24 carbons, originally isolated from toad skin used on Chinese medicine. Bufodienolides shows many biological activities, including anticancer activities. Recently, our group reported the strong cytotoxic potential of several natural and semi-synthetic bufodienolides from toad *R. schneideri* against several human cancer cell lines, and among these studied compounds, hellebrigenin presented highest cytotoxicity. The present study aimed to evaluate the cytotoxic and genotoxic potential of hellebrigenin isolated from *R. schneideri* on HL-60 leukemia cancer cell line. **Methods:** Flow cytometry studies were conducted to evaluate cell viability, interference on cell cycle progression, phosphatidylserine (PS) externalization and caspases (8, 3/7) activation. Comet and chromossomal aberrations tests were performed to evaluate the genotoxic and mutagenic potential of this bufodienolide. All tests were performed using hellebrigenin at 0.03, 0.06, 0.12 µM concentrations for 24h. Doxorubicin (0.6 µM) was used as positive control. **Results and Discussion:** Our data showed hellebrigenin causes a disruption of cell membrane and G2/M cell cycle arrest. This interference on cell cycle progression seems to be irreversible since internucleosomal DNA fragmentation (sub-G1 cell population) and other apoptotic markers (externalized PS and cleaved caspases) were detected in HL-60 cultures treated with hellebrigenin. DNA damages and chromosomal aberrations were not observed. These data confirm that hellebrigenin has anticancer properties, probably to interfere on progression of cell cycle leading cell death. **Support:** CNPq, CAPES, BiotechCell.

10.015 **Cytotoxic potential of the venom of *Crotalus durissus cascavella* in tumor cell lines**. Araújo LS[1], Evangelista JSAM[1], Rosas NSC[1], Conceição ASMM[1], Rocha DD[2], Wilke DV[2], Ximenes RM[2], Guarnieri MC[3], Evangelista JJF[2], Costa Lotufo LV[2] [1]UECE – Ciências Veterinárias, [2]UFC – Fisiologia e Farmacologia, [3]UFPE – Zoologia

Introduction: The basic knowledge about cancer is increasing rapidly, however, advances in the clinical treatment against tumors has been acting not so satisfactory in the control of this disease. For this reason, the development of alternative drugs is relevant in an attempt to improve the prognosis and to increase survival of cancer patients. The snake venoms are natural sources of bioactive compounds with therapeutic potential. The aim of this work is to analyze the cytotoxic effect of the venom of *Crotalus durissus cascavella* (Cdcasca) against tumor cell lines. **Methods:** The cytotoxicity of this venom was analyzed after 72 h of treatment by the MTT (salt 3 - (4,5-dimethylthiazol-2yl) -2,5-difeniltetrazolium bromide) colorimetric assay in the following cell lines: OVCAR-8 and SKOV3 (ovarian carcinoma), PC-3M (prostate carcinoma), MCF-7 (breast carcinoma) and SF-268 (glioblastoma). The cell cycle distribution, plasmatic membrane integrity and cell concentration was also analyzed using flow cytometry after 24 h treatment with 1, 2 and 4 µM of the venom. Data was further analyzed in the Graphpad Prism 5. This project was submitted to the ethics committee with the process number 11516651-3/62. **Results and Discussion:** The results of the MTT assay are expressed as IC_{50} (concentration capable of killing 50 % of the cells) and the values obtained ranged from 2.4 to 6.9 µM, after 72 h of incubation. Among the five cell lines tested, PC-3M and OVCAR-8 were the most sensitive to the venom, with IC_{50} of 2.4 and 2.7 µM respectively. Then, the OVCAR-8 cell line was used to evaluate the effect of the venom on the cell cycle distribution, which showed a discreet arrest at the G_0/G_1 phase when compared to the negative control. Tumor cell lines from benign adenoma pituitary (GH3) and rat glioma (RT2) exports the venom of *Crotalus* genus, showed considerable accumulation in cell subG1 suggesting cell apoptosis and the rest accumulated in G0/G1 (SOARES *et al.,* J. Venom. Anim.Anim. Toxins incl.Incl. Tox.Trop. Trop. Dis. Dis vol.16 no.3 Botucatu 2010, (16), 480, 2010). Membrane integrity assay showed that only when treated with 4 µM of venom cells begin to lose integrity which is related to cell death. Interestingly the number of cells (cells / mL) is already reduced at the concentration of 2 µM of the venom (40.7×10^4) when compared to the negative control (49.4×10^4), showing that the cells are actually not proliferating instead of dying, which is in accordance to the cell cycle arrest. Features of apoptosis were also described in the hamster ovarian carcinoma (CHO-K1), when treated with venom of the genus *Crotalus.*(TAMIETI et al., J. Venom. Anim. Anim. Toxins incl. Incl. Tox. Trop. Trop. Dis. (13), 56, 2007). **Conclusion:** The venom of *Crotalus durissus cascavella* presented a high cytotoxic effect against several tumor cell lines probably due to the interference in the cell cycle of treated cells. However further studies need to be made to show the exact mechanism of action by which this venom is acting. **Financial Support:** CAPES, CNPq and FUNCAP.

10.016 Cytotoxicity and anti-angiogenic activity of a PLA2 from *Bothrops jararacussu* (Jararacuçu) snake venom. Sousa NC[1], Barillas SG[1], Lorenzetti R[1], Ruiz AL[2], Böttcher-Luiz F[3], Carvalho JE[2], Serrano SMT[4], Zelanis A[4], Hyslop S[3] [1]Unicamp – Farmacologia, [2]CPQBA-Unicamp – Farmacologia, [3]CAISM-Unicamp, [4]CAT-CEPID-IBu – Toxinologia Aplicada

Introduction: Snake venoms may stimulate angiogenesis via venom VEGF-like factors, or inhibit this phenomenon by enzymes such as L-amino acid oxidases, disintegrins and phopholipases A_2 (PLA_2). The anti-cancer activity of snake venoms may be mediated by direct cytotoxicty and anti-angiogenic activity. In this work, we examined the cytotoxicity of bothropstoxin-1 (BthTX-1), a Lys49 PLA_2 isolated from *Bothrops jararacussu* snake venom, in cancer cell lines, and assessed its anti-angiogenic activity in an egg-yolk model. **Methods:** BthTX-I was purified from *B. jararacussu* venom by a combination of gel filtration (Superdex 75) and ion exchange (CM-Sepharose) chromatographies; purity was confirmed by SDS-PAGE, RP-HPLC and mass spectromery, and mass spectrometric analysis of tryptic fragments was used to identify the protein. PLA_2 activity was assayed colorimetrically (Price 3rd JA, *J. Biochem. Biophys.Methods* **70,** 441, 2007). Cancer cell lines MCF7, NCI-ADR, OVCAR-03, U251 and VERO were grown under standard conditions and incubated with BthTX-1 (0.25, 2.5, 25 or 250 µg/ml) for 6 h, 24 h and 48 h, after which cell viability was assessed by the methyl thiazolyl tetrazolium (MTT) assay (Vanhée et al., J. Immunol. Methods. **26**, 159, 1993). Anti-angiogenic activity was assayed in embryonated eggs (Kusaka M *et al.*, *Biochem. Biophys. Res. Commun.* **174,** 1070, 1991) and was assessed after 24 h by counting the number of new vessels in the absence and presence of VEGF (4 ng) and bFGF (100 ng). The role of enzymatic activity in the responses to BthTX-1 was examined by pretreating the toxin with *p*-bromophenacyl bromide (*p*-BPB; 0.6 mM, 24 h, 23 ^{o}C). **RESULTS:** BthTX-1 was cytotoxic to all cell lines, although there was considerable variation in the responses among cell types. The toxin concentrations causing total growth inhibition (TGI) were <0.25 mg/ml for U251 cells, 18.7 mg/ml for NCI-ADR cells, 27.1 µg/ml for OVCAR-03 cells, 60.8 mg/ml for MCF-7 cells and 70.7 µg/ml for VERO cells. In chick embryos, 0.125 mg BthTX-1 reduced basal vessel formation by 25% (from 80±4 to 60±5 vessels/microscopic field at 5x magnification, mean±SD, n=8 $p<0.05$, Student´s *t*-test) and 0.5 mg BthTX-1 reduced basal vessel formation by 81% (from 80±4 to 15±3 vessels/field; n=8 $p<0.05$, Student´s *t*-test); higher amounts of BthTX-1 (1 and 2 mg) inhibited angiogenesis to the same extent as 0.5 mg. BthTX-1 (0.5-2 mg) also attenuated VEGF and bFGF-induced angiogenesis by ≥90% and ≥84% respectively (from 178±17 to 18±4 vessels/field and from 140±5 to 20±3 vessels/field in the case of 0.5 mg; n=3 $p<0.05$, Student´s *t*-test). Pretreatment with p-BPB inhibited PLA_2 activity by 100% and abolished the cytotoxicity and inhibitory activity of BthTX-1 on basal and growth factor-stimulated angiogenesis. **Conclusions:** These results show that BthTX-1 is cytotoxic to a variety of cancer cell lines and also has potent anti-angiogenic activity. **Financial support:** CAPES, CNPq, FAPESP.

10.017 Liver enzymatic regeneration is stimulated by association verapamil-amniotic membrane, in partially hepatectomized rats. Bastos WP, Vilela-Goulart MG, Gomes MF CEBAPE-FOSJC-Unesp

Introduction. The liver is almost unique amongst the tissues of the body in its capacity for regeneration after resection. The calcium blocker verapamil has demonstrated to enhance liver regeneration in partially hepatectomized rats and the amniotic membrane accelerates the healing process in surgically damaged liver. The association of verapamil and amniotic membrane on experimental liver regeneration is studied in rats. **Methods**. Ninety six male rats, partially hepatectomized, were divided in groups: a- operated and not treated; b- rats whose liver lesion was dressed with homogenous amniotic membrane; c- rats treated with verapamil; d- rats treated with verapamil plus amniotic membrane. The groups were sacrificed at the 10^{th}, 20^{th}, 30^{th} and 40^{th} days postoperative. Hepatic functional responses were evaluated in plasma and liver tissue by the enzymatic activity of aspartate and alanine aminotransferases, alkaline phosphatase and gamma-glutamyltransferase. Enzymatic reference values were determined in not operated or treated rats. **Results and Discussion**. All the enzymes significantly raised after the surgery, returning to normal in the liver at the 40^{th} day in the verapamil and verapamil plus membrane amniotic groups. The enzymatic levels along the experiments were very significantly lower in the two verapamil treated groups than in not treated. These results evidence an important protective effect of verapamil, which could be attributed to a lowered intracellular calcium induced by the calcium blocker, since it has been demonstrated that an increasing the cytosolic calcium concentration can induce a variety of phenomena that lead to liver cell lesion and death. The protective response of amniotic membrane, not alone, but in presence of verapamil, could be attributed to the membrane property of enhancing wound healing in various tissues and liver. **Conclusion**. The amniotic membrane was shown to exert a protective effect of liver enzymatic regeneration, after partial hepatectomy, in verapamil treated rats. **Financial support by** FAPESP, Grant 2004/08656-3. Approved by Ethics Committee for Animal Use, 0505/2003 Protocol.

10.018 **Evaluation of antitumor activity of molecules derived from the isothiocyanate**. Guerra FS[1], Boylan B[2], Radulovic N[3], Fernandes PD[4] [1]LAFION-UFRJ, [2]Panoz Institute-Trinity College – Pharmacy and Pharmaceutical Sciences, [3]University of Ni – Chemistry, [4]ICB-UFRJ

Introduction: The isothiocyanates, such as sulforaphane and phenethyl isothiocyanate demonstrated to inhibit carcinogenesis and tumorigenesis, being useful substances against the development and spread of tumors. It has been shown that phenethyl isothiocyanate induces apoptosis in some cell lines and, in some cases, can induce apoptosis in cells which resistance to chemotherapeutic agents currently in use (Zhang et al., 2005). Therefore, the search for new compounds, leading to the identification of substances more to target for types individualized of cancer with a lower incidence of collateral effects, less toxicity to normal cells and lower cost, is an important strategy for the search for new drugs against the cancer, and this is the objective of this work.
Methods: The cytotoxicity of the analogs benzyl isothiocyanate (BZI), Furanyl isothiocyanate (FI), and n-Butyl isothiocyanate (BI) were evaluated through the mitochondrial respiration based on the metabolic reduction of the bromide of 3-(4,5-dimethylthiazol-2-yl)-2,5-difenltetrazolium (MTT) to formazan. The cells lines K562 and Lucena (10^4 cells/well) were incubated with the substances in concentrations 1, 10 and 30 µg/ml for 72 hours. Statistical analyzes were performed by ANOVA with Bonferroni post-test (* $p < 0.05$). **Results:** The analog BZI reduced cell viability in K562 cells in 31.9% and 23.3% (10 µg/mL and 30 µg/mL, respectively). In the cell line Lucena, cell viability was reduced was 32.1%, 41.1% and 24.4% (1 µg/mL, 10 µg/mL and 30 µg/mL, respectively). The analog FI reduced cell viability in K562 cells 30.1% and 27.1% (10 µg/mL and 30 µg/mL , respectively). In the cell line Lucena the cell viability was reduced in 29.5%, 45.2% and 47.8% (1 µg/mL, 10 µg/mL and 30 µg/mL , respectively). The analog BI reduced cell viability of K562 in 11.4% and 18.1% (10 µg/mL and 30 µg/mL , respectively). In the cell line Lucena the cell viability was reduced in 32.1% and 33.9% (1 µg/mL and 10 µg/mL, respectively). **Conclusions:** The results showed that the isothiocyanate derivatives tested are potent inhibitors of cell viability in leukemic cell lines tested, even the line resistant to multiple drugs such as Lucena. **References:**
Zhang, Y.; Li, J.; Tang, L. Free. Radical Biol. Med., 38: 70-77, 2005. **Financial support:** CAPES, CNPq, FAPERJ, and IVB

10.019 Reversion of multidrug resistance by apiole-doxorubicin association in NCI/ADR-RES ovarian cancer cell line. Longato GB[1,2], Monteiro PA[1,2], Ruiz ALTG[1], Foglio MA[3], Carvalho JE[1] [1]CPQBA-Unicamp – Farmacologia e Toxicologia, [2]IB-Unicamp, [3]CPQBA-Unicamp – Fitoquímica

Introduction: Multiple-drug resistance is the major clinical obstacle in cancer therapy. The use of drug combinations to circumvent tumour resistance is a well-established principle of cancer therapy and multiple agent chemotherapy which includes vincristine and doxorubicin has been reported to be more effective than single agent therapy[1]. Recent pharmacological studies have reported the ability of several calcium channel blockers to reverse the resistance of natural product chemotherapeutic drugs *in vitro* and *in vivo.* Being the apiole a calcium channel blocker[2], this study aimed to evaluate its capacity in reversing the multidrug-resistance of ovarian cancer cell line (NCI/ADR-RES), in association to doxorrubicin. **Methodology:** Apiole was isolated from *Piper regnellii* (Miq.) C.DC. var. regnellii leaves. *In vitro* antiproliferative activity of apiole was evaluated in association to doxorubicin in human ovarian tumor cell line NCI/ADR-RES by sulforhodamine B colorimetric assay[3]. Samples concentrations ranged from 0.025 to 25 µg/mL for doxorubicin and 2.5 to 250 µg/mL for apiole. After 48 hours of treatment, the antiproliferative activity was determined through GI_{50} (50% of growth inhibition) values and the graphics were constructed. **Results:** Table 1. GI_{50} values (µg/mL) of doxorubicin administered alone and in association with apiole.

Doxo	Apiole 250 µg/mL + Doxo	Apiole 25 µg/mL + Doxo	Apiole 2,5 µg/mL + Doxo
2.57	<0.025	0.30	3.25

In the presence of apiole at concentration of 250µg/mL, the GI value of doxorubicin was reduced 100 times (from 2.57 to 0.025 µg/mL) and 10 times (from 2.57 to 0.30 µg/mL) in the presence of apiole at concentration of 25µg/mL. Apiole at concentration of 2.5 µg/mL did not reverse response, demonstrating a huge correlation between drug concentration and effectiveness. **Discussion:** The combined therapy with apiole was efficient in promoting doxorubicin-resistance reversion. A major mechanism of multidrug resistance has been attributed to enhanced drug efflux, resulting in reduced intracellular drug accumulation. This is mostly caused by an increased expression of the MDR7 gene, which encodes P-glycoprotein (P-gp), an energy-dependent transmembrane efflux pump[4] presented in NCI/ADR-RES cell lines. Several calcium channel blockers act reversing the resistance of natural product chemotherapeutic drugs *in vitro* and *in vivo*, by interacting to this protein[5]. Apiole is probably serving as a substract to P-glycoprotein, inhibiting the doxorubicin efflux. Further studies are in progress in our laboratory to verify the mechanisms of action of the doxorubicin-apiole association in experimental in vivo cancer models. **References:** 1. Dancey JE. Nature Rev. Drug Disc, 5:649, 2006. 2. Neuhaus-Carlisle K. Phytomed, 4:67,1997. 3. Skehan P. J. Nat. Cancer Inst, 82:1107, 1990. 4. Chaudhary PM. Cell, 66:85, 1991. 5. Chiu LY. Toxicol. Lett, 192:408, 2010. We thank Fapesp, Capes and CNPq for the finacial support.

10.020 **Antimelanoma activity of a tetrahydrofuran derivative of α-lapachone**. Santos EA[1], Ferreira SB[2], Pessoa C[1], Moraes MO[1], Kaiser CR[2], Ferreira VF[3], Costa-Lotufo LV[1], Montenegro RC[4] [1]UFC – Fisiologia e Farmacologia, [2]UFRJ – Química Orgânica, [3]UFF – Instituto de Química, [4]UFPA – Ciências Biológicas

Introduction: Quinones represent a class of secondary metabolites widely distributed in nature that have diverse pharmacological activities, emphasizing microbicidal, anti-inflammatory, antitumor and antimetastatic properties. Among the naphthoquinones, α-lapachone proves to be a good prototype for the development of new drugs to treat multiple drugs resistant tumor cells (MDR) and also to inhibit angiogenesis. The aim of the present work was to evaluate the *in vitro* antimelanoma activity of α-lapachone and its tetrahydrofuran derivative. **Methods**: The cytotoxicity was evaluated against B-16 (murine melanoma), B-16-F-10 (murine melanoma), MALME-3M (metastatic human melanoma), and M14 (human melanoma) cancer cells by the MTT assay. The cell migration assay was performed in B-16-F-10 cell line at concentrations of 0.1, 0.25 and 0.5 μM, previously determined by colony formation assay. **Results and Discussion**: The compounds induced cytotoxicity in all tested melanoma cells with IC_{50} values ranging from 5.9 μM in B16-F10 to 9.7 μM in M14 after 72 hours of incubation. The tetrahydrofuran derivative showed IC_{50} value of 13.3 μM after 24 hours of exposure in B-16-F-10 cells. In the cell migration assay, there was a significant decrease in cell motility in concentrations of 0.25 and 0.5 μM. **Conclusion**: The results suggest that the introduction of the tetrahydrofuran group in α-lapachone molecule increases the cytotoxic activity in most tested tumor cells, while it also confers inhibitory properties on the cell motility in metastatic melanoma model, suggesting that this molecule may present antimetastatic activity. **Supported by**: FUNCAP, CAPES, CNPq, PRONEX.

10.021 **Antiproliferative effect of *Agaricus brasiliensis* mycelium *in vitro***. Navegantes KC[1], Albuquerque RFV[1], Santa-Hutz HD[2], Gomes RS[1], Monteiro MC[1] [1]FF-UFPA, [2]Unicentro – Engenharia de Produção

Introduction:*Agaricus brasiliensis* or also denoted *Agaricus subrufescens* is native fungus to Brazil and popularly known as the mushroom of the sun, which can be extracted from the mushroom fruiting bodies, mycelium and/or broth of submerged culture. The mushroom has been widely used as a nutraceutical, because their pharmacological properties, such as antimetastatic, antitumoral and immunostimulating. Recently, ours group showed the dietary supplementation with *A. brasiliensis* mycelium (LPB) prevented loss of body weight, inhibited tumour growth, induced the increase of CD4 T cells and CD25CD4 T subsets in peripheral organs and downregulated TNF-alpha production in plasma in mice. Thus, this study aimed to evaluate the effect of the LPB in proliferation of splenocytes induced by a mitogen Concavalin A (con A). **Methods:** All steps involved in the maintenance of *A. brasiliensis* (LPB03) were described previously by Santa et al., 2010. All experiments were conducted in accordance with NIH guidelines on the welfare of experimental and with the approval of the Ethics Committee of the Federal University of Paraná (N.70). The viable cell was performed trypan blue 0.4%, and to proliferation was used spleen cells from BALB/c mice at a density of $1x10^6$ cells/ml were incubated or not with con A (5µg/mL) in presence of LPB (6.3; 3.1 and 1.56 µg/mL) in 5% CO2 at 37ºC for 24 or 48 hours. Ten microlitres of methylthiazoletetrazolium (MTT 5 mg/mL) was added 4 h prior to the determination of index proliferative, each group was performed in three replicates. Moreover, to proliferation and dead cells also were performed the flow cytometric analysis, for this, splenocytes were treated as described above and washed with FACS-PBS, centrifugated and resuspended in citrate buffer with propidium iodide (PI) solution. The DNA content in each cell nucleus and forward scatter (FSC) and side scatter (SSC) were set to 10^4 cells were gated using CellQuest software (FACSCalibur Flow Cytometer). Values were expressed in index proliferative and percentage of dead cells. Statistical Analysis was used one-way ANOVA,* $p \leq 0.05$. **Results and Discussion:** To proliferation assay for MTT, ours data showed that the LPB was able inhibit the proliferation induced by con A after 48 hours (CON A= 1.23 ± 0.286; LPB 6. 25 µg/mL = 0.89 ± 0.076, EPS3.12 = 0.93 ± 0.256). In addition, the flow cytometric analysis also showed that the LPB had antiproliferative action after 48 hours of incubation with splenocyte (CON A= 70.0%; LPB 6. 25 µg/mL = 63.5%, LPB3.12 = 65.0%) and it protected these cells of the death (CON A= 3.7%; LPB 6. 25 µg/mL = 1.9%, EPS3.12 = 2.5%). The finding was confirmed that in lower concentrations of LPB (µg/mL) were observed an antiproliferative effect and protected the spleen cells from death. Recently, we related the antiproliferative action and reduced the NO production of LPB in higher concentration (mg/mL), however, this concentration of LBP did not protect the macrophages from death induced by *C. albicans*. **Financial support:** CAPES/CNPq, FAPESPA-PA; UNIVERSAL/CNPq, UFPA.

10.022 Essential oil of lemongrass (*Cymbopogon citratus*) is cytotoxic to SK-MEL147 (human melanoma cells). Villaverde JM[1], Sanches LJ[2], Luiz RC[3] [1]UEL –Biology Applied to Health Sciences, [2]UEL –Experimental Pathology, [3]UEL – Sciences of Pathology

Introduction: Lemongrass essential oil (*Cymbopogon citratus*) (LGEO) is used in foods flavoring, aromatherapy, and cosmetics industry (GANJEWALA & LUTHRA, 2010). Citral (65-85%), geraniol and β-myrcene are important components of LGEO (Marongiu *et al*., 2006). Citral upregulates p53 and caspase 3 expressions (Dudai *et al*., 2005), and geraniol presents antiproliferative effects against human colorectal cancer (CaCo-2), breast cancer (MCF-7), and hepatoma (HepG2) cells (Carnesecchi *et al*., 2001; Duncan *et al*., 2004; POLO & DE BRAVO, 2006). Based on these properties we evaluated the cytotoxicity of LGEO in human melanoma (SK-MEL 147) and keratinocyte (HaCaT) cells. **Methods:** The cells were cultivated in RPMI medium (10% fetal bovine serum, 1% penicillin/streptomycin mixture), humidified atmosphere, 5% CO_2, 37ºC. Cells were seeded at a density of 5 x 10^4 cells/well in 24 wells plates. Doubling time calculation, Trypan blue exclusion assay, MTT assay, Lactate dehydrogenase release assay were used to cytotoxicity evaluation. Acridine orange/Ethidium bromide staining was used to identify cell death patterns. Experiments were performed in triplicate, three independent repetitions. Tween 80 (2%) e Pro-Lipo® Duo (1% final concentration in culture) were used to prepare LGEO emulsification for cell treatment. Hydrogen peroxide 100µM was used as cytotoxic control. **Results:** All tested concentrations (0.05, 0.1, 0.25, 0.5 and 1 µl of extract per ml of culture medium) reduced SK-MEL 147 viability within 24 hours of treatment. LGEO 0.5 µl /ml demonstrated an increase in doubling time, and higher concentrations induced 100% of cell death after 48h of treatment, making impossible doubling time calculation. For HaCaT cells, no cytotoxic or cytostatic effects were observed. The MTT assay revealed that LGEO is able to reduce cell viability in SK-MEL 147 in a dose-dependent manner (IC_{50} 24h = 0.4 µl/ml). The same concentrations of LGEO showed no ability to reduce cell viability in HaCaT cells. Measure of LDH released in culture medium also showed cytotoxic effect in SK-MEL 147, but no effect in HaCaT cells. LGEO was able to promote apoptosis (from 0.05 to 0.25 µl/ml), necrosis (all concentrations) and autophagy (0.5 e 1 µl/ml) in SK-MEL 147 cells, but showed no death induction in HaCaT cells. **Discussion:** Koffi et al. (2009) demonstrated a cytotoxic effect againstHaCaT for high concentrations of LGEO (IC_{50} 24h = 150 µl/ml). For SK-MEL 147 our work is the first report on LGEO cytotoxicity and autophagy induction. Citral is able to induce G2/M phase cell cycle arrest, caspase-3 activation, and dose-dependent apoptosis and necrosis(Dudai et al., 2005; Chaouki et al., 2009). SK-MEL 147 is a p53 wild type (BERTRAND *et al*., 2007), and HaCaT is heterozygous mutant (LEHMAN *et al*., 1993), these genetic differences could be related to observed responses for LGEO, once p53 protein is involved in cell cycle arrest and apoptosis induction (RYAN *et al*., 1993). Our results demonstrate that LGEO can be a source of products having chemopreventive activity against human melanoma. **Acknowledges:** By Samya® Aromaterapia for providing LGEO.

10.023 **Role of bacterial translocation in the pathogeneses steatohepatitis induced by irinotecan.**

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Introduction: Nonalcoholic steatohepatitis (NASH) is a new complication of irinotecan (IRI)-based anticancer regimens. NASH may complicate the clinical course of patients submitted to hepatic resection because it decreases hepatic function reserve. Recently, we developed a new experimental animal model of the IRI-induced steatohepatitis with the full histological findings seen in the human NASH. However, the pathophysiology of IRI-related NASH remains to be clarified. Then, we aimed to investigate the role of bacterial translocation and expression of inflammatory markers involved in IRI-induced NASH pathogenesis. **Methods:** Swiss mice (n=8, 23-25g) were divided into groups and injected Saline (5ml/kg, ip) or IRI (50 mg/kg, ip, 3x/week/7weeks). After seven weeks, peripheral blood was collected from the retro-orbital plexus to measure serum enzymes ALT and AST (U/L) and proteins. The animals were killed by cervical dislocation and liver and intestine were removed, weighed (g/30 g body weight of animal) and carefully dissected for histopathological analysis by the Kleiner scores (lobular inflammation [0-3], steatosis [0-3] and vacuolization [0-3]), measurement of myeloperoxidase activity (MPO, U/mg tissue) and lipid content, immunohistochemical assay for Interleukin-1 (IL-1β), inducible nitric oxide synthase (iNOS) and Toll-Like Receptors 4 (TLR4). Portal and systemic blood samples were cultured in gram negative-selective medium to determine bacterial translocation. Intestinal histopathologic analysis was also performed. $P<0.05$ was accepted. Data were analyzed through ANOVA/ Student Newman Keul or Kruskal Wallis/Dunn as appropriate. CEPA: 21/12. **Results:** IRI significantly increased liver wet weight (1718±156.2) versus saline group (1110± 66.1). IRI caused a marked increase in serum ALT (95.99±4.13) and AST (138.7±9.52), proteins (3.25±0.01), liver MPO (2.19±0.56), lipids (1.07±0.12) and Kleiner's scores (4[4-7]) when compared with saline group (ALT: 44.58 ± 12.03, AST: 84.16 ±8.122; proteins: 5.38 ± 0.25; MPO: 0.06±0.03; lipids (0.27±0.13); Kleiner scores: 1[0-1]. The immunohistochemical analysis in liver samples of IRI-injected group showed a significant increase in IL-1 2[1-3], iNOS 2[2-3] and TLR4 3[1-3] immunostaining compared to saline group IL-1: 0[0-1]; iNOS: 1[1-2]; TLR4: 1[1-2]. Bacteremia was also evidenced in IRI-injected group (portal blood-80%) and (systemic blood-40%) versus saline group (0%). Shortening of the villi and the alteration of crypt size and architecture were observed in IRI administered mice (4[3-4]) compared with control group (0[0-0]). Immunohistochemical analysis in intestinal samples of IRI-injected group showed a significant increase in IL-1 2[1-3], iNOS 1[1–2] and TLR4 2,5[0–3] immunostaining compared to saline group (IL-1: 0[0-1]; iNOS: 0[0-1]; TLR4: 1[0–2]**Conclusion:** These results indicate that IRI causes rupture of gut barrier and intestinal bacterial translocation that could trigger liver inflammation and steatohepatitis development. **Financial support: FUNCAP,** CAPES, CNPq.