P11-038
Phytochemical, antioxidant and mitochondrial permeability transition analysis of fruit skin ethanolic extract of *Annona muricata* Linn. (Soursop)

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Question: *Annona muricata* Linn. contains a group of bioactive long chain fatty acid derivatives called Annonaceous acetogenins, which have shown selective cytotoxicity against several cancer cell lines and other abnormal cells by a known mechanism. The current study analyzed the phytochemical and antioxidant properties of the fruit skin ethanolic extract of *Annona muricata* (ESA) and its effect on the opening of rat liver mitochondrial membrane permeability transition (MMPT) pore *in vitro*. *Methods*: Tests for the phytochemical constituents of the extract and antioxidant assays were carried out following standard protocols while the opening of the MMPT pore in the presence of varying concentrations of the extract was spectrophotometrically assayed under succinate-energized conditions. Calcium chloride ($CaCl_2$) solution and spermine at specified concentrations were employed to trigger and inhibit MMPT pore opening respectively. *Results*: The results show that terpenoids, steroids and glycosides were found present in the fruit skin ethanolic extract and the extracts were found to have very low antioxidizing properties at the tested concentrations based on the diphenyl-1-picryhydrazy (DPPH) radical scavenging activity assay. Lipid peroxidation was induced in a concentration-dependent manner on both the cytosolic and mitochondrial hepatocyte fractions *in vitro*. In the absence of triggering agent, 0.84 mg/ml concentration of ESA induced the opening of the pore by 129% whereas the other three lower concentrations (0.12 mg/ml, 0.36 mg/ml and 0.60 mg/ml) did not induce MMPT pore opening. In the presence of the triggering agent, the extracts showed no inhibitory activity; rather there was a greater increase in the induction with increasing concentration of extracts. *Conclusions*: From the foregoing, the fruit skin ethanolic extract of *Annona muricata* may possibly contain bioactive components that are likely to induce apoptosis thereby adding to the growing list of nature-friendly chemopreventive and curative therapy of cancer.

http://dx.doi.org/10.1016/j.toxlet.2015.08.1099

P11-039
The placenta in toxicology: Observed effects of breast cancer treatment

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Purpose: Pregnancy and breast cancer is a rare coincidence, but breast cancer is one of the most common malignancies during pregnancy. Guidelines on treatment for breast cancer during pregnancy already exist and demonstrate that pregnancy-associated breast cancer (PABC) can be treated according to recommendations for non-pregnant women with breast cancer. Nonetheless, a decreased birth weight is often observed in newborns which may be due to harmful effects of chemotherapy on the placenta. Therefore, we aim to determine toxicity of commonly applied chemotherapeutic drugs. *Methods*: Placental villous tissue explants (PVTE) were obtained after spontaneous delivery or cesarean section following normal pregnancy ($n = 3$). PVTE were incubated after a recovery time of 24 h with doxorubicin, docetaxel, 5-fluorouracil or vincristine for at least 48 h. Supernatant analyses of glucose, lactate, lactate dehydrogenase (LDH), human chorionic gonadotropin (hCG), estrogen and progesterone were performed. The metabolic activity was evaluated *via* MTS assay. Data were analyzed with SPSS 22 using a linear mixed model. Furthermore, PVTE were embedded in paraffin for histological examinations. *Results*: Glucose, lactate and LDH as potential markers of toxicity were significantly affected in the supernatant of PVTE treated with the different chemotherapeutics. In contrast, expression of hCG, estrogen and progesterone was not modified. The metabolic activity of PVTE evaluated *via* MTS assay was only reduced by doxorubicin and docetaxel, but not by 5-fluorouracil and vincristine treatment. Hematoxylin/eosin staining of 5-fluorouracil and vincristine treated PVTE showed more morphological anomalies than doxorubicin and docetaxel treatment. *Conclusion*: PVTE display a suitable tool for studying effects of different chemotherapeutic drugs during pregnancy. Among the analyzed parameters, only the metabolic markers glucose, lactate and LDH and the MTS assay appear to be useful for detecting harmful effects and for further investigations. Hematoxylin/eosin staining indicates early toxic morphological changes, also when the MTS assay did not. Immunohistochemical staining may further increase sensitivity.

http://dx.doi.org/10.1016/j.toxlet.2015.08.1100

P11-040
Evaluation of cytotoxicity and inhibitory effects of cervical cancer treatment in 2D and 3D cell culture models

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Purpose: Cervical cancer with an estimated incidence of 0.1–12 per 10,000 pregnancies is the most common solid cancer diagnosed in pregnancy. Besides surgery, neoadjuvant chemotherapy used in the second and third trimester seems to be a promising strategy to avoid rapid tumor progression while maintaining pregnancy. However, such treatment is hampered by quiescent tumor cells featuring a high resistance against chemotherapeutics. Since these cells also occur in multicellular tumor spheroids (MCTS), we used this *in vitro* model to study the effects of chemotherapeutics in 3D cell cultures compared to 2D. *Methods*: The two well established cervix carcinoma cell lines SiHa and SW756 were incubated with cisplatin, paclitaxel, topotecan and bleomycin in three different concentrations. Firstly, potential toxic effects of the treatment were analyzed by MTS assay in 2D cell culture. To generate a 3D *in vivo* tumor model, cells were cultivated *via* hanging drops amended with 25% methocel. After a period of three days, spheroids were incubated with the drugs for 24 h in a 0.5% poly-HEMA coated 96-well plate. The cytostatic drug effects were evaluated microscopically. *Results*: In the conventional 2D model, MTS analysis demonstrated in both cell lines a decreased metabolic activity induced by chemotherapeutics. Regarding the establishment of a 3D model SiHa cells did not form spheroids. Therefore, only SW756 spheroids were used for further investigations. Modified shape and reduced size of the spheroids were detected after incubation with cisplatin and paclitaxel. After 24 h, spheroids lost their typical shape and transformed into loose aggregates. In contrast, topotecan and bleomycin did not influence spheroid morphology while the majority of cells grown in 2D were eliminated. *Conclusion*: Cisplatin and paclitaxel harm the cervical cancer cell lines SiHa and