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BOOK OF ABSTRACTS

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OBJECTIVES:

The study aimed to analyze the time-dependent consequences of stress on gene expression responsible for diurnal endocrine Leydig cell function connecting them to the glucocorticoid-signaling.

METHOD / DESIGN:

Three *in vivo* and one *ex vivo* experimental approaches were applied: (1) to examine the effect of Imobilization sress (IMO) on Leydig cell activity, IMO was applied from ZT0-3 followed by the expressional/functional study in hours after IMO (ZT3, ZT11, ZT17, and ZT23); (2) to prove that glucocorticoids mimic IMO effects rats were treated orally with Dexason at ZT0 and effects were analyzed after treatment in ZT3, ZT11, ZT17, and ZT23 respectively; (3) to estimate glucocorticoids contribution in the effects of stress, testes of IMO rats were treated by RU486 (glucocorticoid receptor blocker) (4) to obtain information about direct effect of glucocorticoids on clock and steroidogenesis in Leydig cells, *ex vivo* experiments were performed using primary cultures of purified Leydig cells.

RESULTS:

In the first 24h after the stress event, a daily variation of blood glucocorticoids increased, and testosterone decreased; the reduced testosterone/corticosterone detected were lowest at the end of the stress session overlapping with inhibition of Leydig cells' steroidogenesis-related genes (*Nr3c1/GR, Hsd3b1/2, Star, Cyp17a1*) and changed circadian activity of the clock genes (the increased *Bmal1/BMAL1* and *Per1/2/PER1* and decreased *Cry1* and *Rev-erba*). The glucocorticoid-treated rats showed a similar response. The PCA displayed an absence of significant differences between treatments especially *Per1* and *Rev-erba*. This observation was confirmed by the in vivo blockade of the testicular glucocorticoid receptor during stress and *ex vivo* treatment of the Leydig cells with hydrocortisone and glucocorticoid receptor blocker.

CONCLUSIONS:

In summary, stressful stimuli can entrain the clock in the Leydig cells through glucocorticoid-mediated communication.

T3-P-57 *In vitro* and *in silico* investingation of antimicrobial activity of essential oils from two *Pastinaca Sativa* subspecies

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KEYWORDS: *Pastinaca sativa subsp. sativa*; *Pastinaca sativa subsp. urens*; essential oils; microdilution method; molecular docking.

INTRODUCTION:

Cultivated parsnip (*Pastinaca sativa* subsp. *sativa L.*, Apiaceae) root is a well-known vegetable, common ingredient of soups,

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stews, salads, casseroles etc. Besides, its leaves and young shoots can be added to soups and fruits are used as a condiment. Furthermore, young shoots of wild-growing parsnips, e.g. *P. sativa* subsp. *urens* (Req. ex Godr.) Čelak., are consumed pickled or in salads or soups and it is considered that their essential oil acts as a natural preservative.

OBJECTIVES:

To investigate and compare the antimicrobial activity of the essential oils obtained from the roots, leaves, stems, flowers and fruits of cultivated *P. sativa* subsp. sativa and wild-growing *P. sativa* subsp. urens collected in Serbia. Furthermore, the most active essential oil constituents (against the most susceptible microorganisms) were predicted *in silico*.

METHOD / DESIGN:

Minimum inhibitory concentrations (MICs) and minimum bactericidal/fungicidal concentrations (MBCs/MFCs) of the essential oils (isolated by hydrodistillation using Clevenger-type apparatus) were determined by microdilution method against three Gram-positive bacteria: *Staphylococcus aureus* ATCC 11632, *Bacillus cereus* clinical isolate and Listeria monocytogenes NCTC 7973, three Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Salmonella Typhimurium* ATCC 13311 and *Enterobacter cloacae* ATCC 35030, three *Candida* standard strains: *C. albicans* ATCC 10231, *C. tropicalis* ATCC 750 and *C. parapsilosis* ATCC 22019, and three *Candida* isolates from oral cavity: *C. albicans* 475/15, *C. krusei* H1/16 and *C. glabrata* 4/6/15. Pharmacokinetic properties of the compounds present in at least one oil in the quantity ≥ 1% (determined by GC-FID and GC-MS) were initially evaluated using SwissADME web tool and molecular docking was performed using AutoDock Vina 1.1.2 (interactions were visualized using Discovery Studio Visualizer 2019).

RESULTS:

All the investigated essential oils of the two Pastinaca sativa subspecies were able to reduce the growth of different tested *Candida* strains (MIC range 0.25-2 mg/mL; MFC range 0.5-4 mg/mL). The most promising activity was observed for both root oils (MIC range 0.25-1 mg/mL; MFC range 0.5-2 mg/mL). Among investigated *Candida* strains, *C. parapsilosis* strain was the most sensitive to these essential oils (MIC range 0.25-1 mg/mL; MFC range 0.5-2 mg/mL). The antibacterial activity of the tested essential oils was lower compared to their anticandidal potential (MIC range 1-4 mg/mL; MBC range 2-8 mg/mL). Thirty compounds were present in at least one oil in the quantity ≥ 1%. Estimation of pharmacokinetic properties using SwissADME tool suggested that 23 of these compounds are inhibitors of some of the cytochrome P450 system isoenzymes. This fact led to assumption that they could also act against fungal sterol 14α-demethylase (CYP51), which is a common target of antifungal drugs (e.g., ketoconazole). Thus, the compounds (3D structures downloaded from PubChem) were docked to the active site of this enzyme (downloaded from Protein Data Bank, PDB code 5TZ1). The highest affinities were predicted for sesquiterpenes caryophyllene oxide, (E)-caryophyllene, germacrene D, α-copaene, β-bourbonene and δ-cadinene (free binding energies from -9.4 to -8.7 kcal/mol; ketoconazole -11.6 kcal/mol). These compounds were present in somewhat lower quantities in the essential oils (≤ 9.9%). For dominant compounds of the tested essential oils, e.g. myristicin, γ-palmitolactone and octyl butanoate a bit lower affinities were predicted (free binding energies from -7.3 to -5.8 kcal/mol). Tested compounds mostly docked near the heme of the enzyme and formed hydrophobic interactions with the amino acid residues of the active site. According to SwissADME tool, four of five most active compounds have low absorption from gastrointestinal tract and higher skin permeation value, while caryophyllene oxide and three dominant compounds have high absorption and lower skin permeation value (similarly to ketoconazole).

CONCLUSIONS:

Investigated parsnips represent sources of essential oils and compounds with anticandidal activity.

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