Using precision-cut liver slices (PCLS) to study effects of produced water (PW) extract fractions on cyp1a biomarker gene expression

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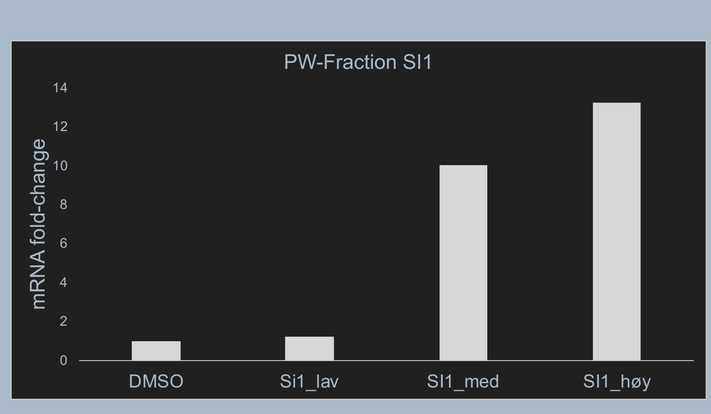
Introduction

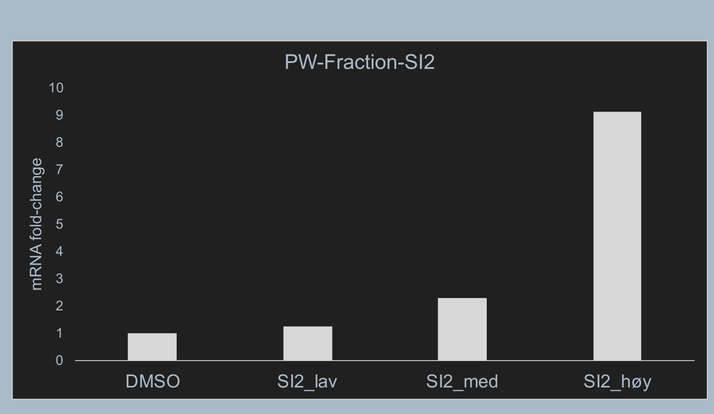
- The marine environment can be polluted by a range of pollutants. Produced water (PW) released from offshore oil installations can pollute the marine environment and affect fish and other organisms. Biomarkers such as cyp1a gene transcript and protein levels in fish are used to assess exposure fish to certain environmental chemicals such as crude oil components (Goksøyr and Förlin, 1992). The aim of this project is to assess the effects of PW extract fractions in Atlantic cod precision-cut liver slices (PCLS) culture using qPCR for cyp1a biomarker gene.

Material and Methods

- PCLS preparation was performed as described in (Eide et al., 2014). Briefly, freshly dissected cod liver blocks were cut into 250 µm slices using Leica vibrating blade microtome and cultured for 48 h in L-15 medium supplemented with 10% fetal bovine serum and antibiotic solutions in a 24-well plate and exposed to either PW fraction or DMSO vehicle . Cell viability was assessed using LDH assay (Roche). RNA extraction was performed using TriReagent (Sigma) and cDNA synthesis, while qPCR was performed using Biorad kits. Cyp1a and acb2 (reference gene) qPCR was performed as described in (Yadetie tel., 2018).

Results





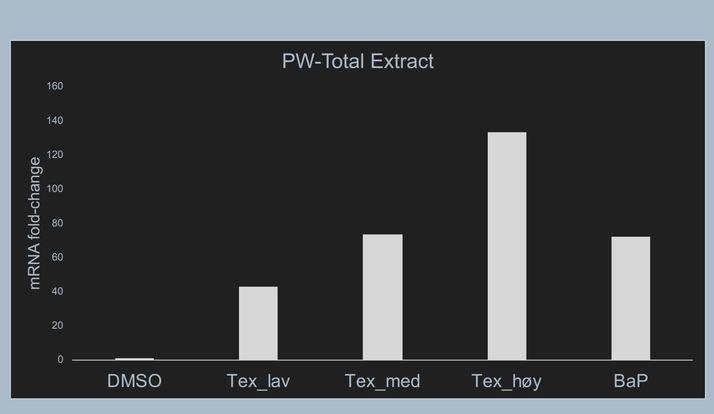
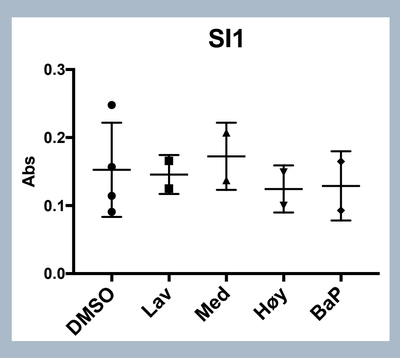
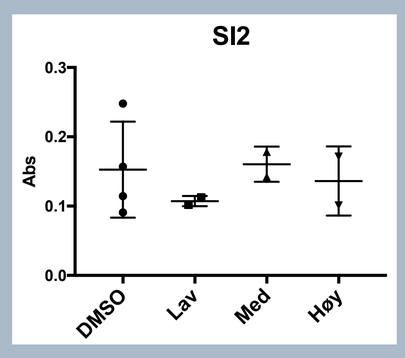
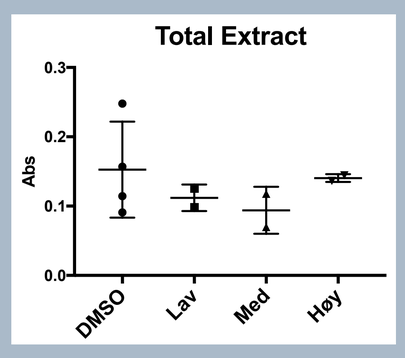
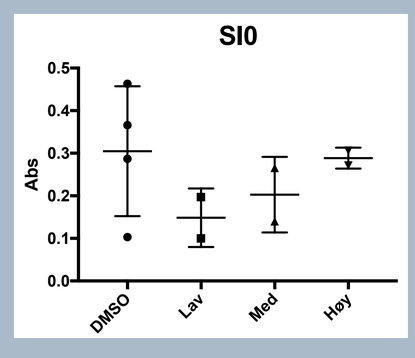


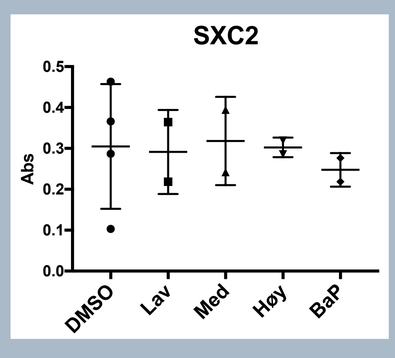
Figure 1 (a, b, & c) : qPCR-assay for cyp1a expression in DMSO control, (low, medium, high) extracts exposed PCLS .











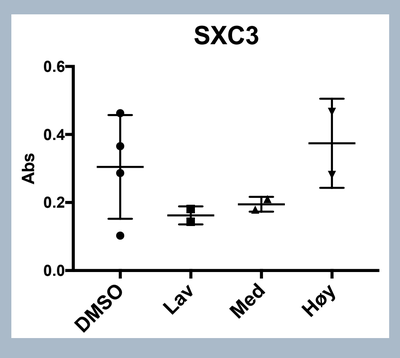


Figure 2 (a, b, c, d, e & f) : Cell viability using LDH assays in DMSO control and PW extract fraction (low, medium and high concentration) exposed PCLS

Summary and conclusions

- The various doses of the extracts tested did not have significant cell toxicity as shown by LDH assay.
- The two fractions as well as the total extract showed strong induction of cyp1a at the highest dose.
- The potency of the extracts may be compared as SI2 < SI1 < Total Extract.

  PW components induce cyp1a in PCLS suggesting possible harmful effects to fish wild close to oil installations.

Future Work

- To test all fractions (approx. 13) with a larger sample size for statistical analysis.
- Test more biomarker genes such as: biomarkers for estrogenic compounds (eg. Vitolligin)

References

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