In vitro screening assay using the murine pre-adipocyte cell line 3T3L1 to study anti-obesogenic activities of chemical compounds

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Obesity is a global health threat with several etiologies. Standard obesity treatment (e.g., bariatric surgery, healthy lifestyle promotion) may not be enough to treat obese people that represent more than half of the European Union population [1]. Model cell culture systems have been vital in obesity studies. The 3T3-L1 cell line [2] has been widely used for the study of bioactive natural compounds research, including research of anti-obesity properties of several natural compounds as fucosterol and phloratannins from the brown algae *Ecklonia stolonifera* [3,4], meridianins from the tunicate *Aplidium meridianum* [5], and extracts from the dandelion *Taraxacum officinale* [6]. Cyanobacteria, known as blue-green algae and producer of cyanotoxins, have shown high content in secondary metabolites with relevant activity (e.g., antibiotic, antifouling, anticancer) [7]. We are currently exploring the chemical richness of these prokaryotes by testing cyanobacterial strains regarding their anti-obesogenic activity in (pre)adipocyte cells.

Various cyanobacterial strains were grown and their extracts were produced through simple vacuum filtration of their liofilized mass suspended in a mixture of Dichloromethane and Methanol (2:1). These extracts were then fractioneted using vacuum liquid chromatography.

Bioactivity of the fractions was tested in the proliferation assay of preadipocyte cells. Proliferation can be assessed by the Sulforhodamine B (SRB) staining and the incorporation of Bromodeoxyuridine (BrdU) into the DNA. Furthermore, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT bioassay) delivers information about the cellular viability and metabolic activity, through the reduction of MTT to formazan by mitochondrial activity.

The most polar fraction (eluded with 100% MeOH) of the cyanobacterial strain *Planktothrix planctonica* has shown the strongest effect on cell proliferation (above 50% compared with the solvent control). The same was verified for the MTT assay, where the activity of mitochondrial enzymes was increased. After consequent sub-fractionation, purified fractions were obtained and the most polar fraction (fraction 7) showed once again pro-proliferative activity through SRB and MTT assay. Further column chromatography will allow us to purify the single compound responsible for this activity and enable us to elucidate its chemical structure.

References:

[1] OECD (2012), Health at a Glance: Europe, OECD Publishing, Paris, pp. 154.

[2] Green, H. and Meuth, M. (1974), An established pre-adipose cell line and its differentiation in culture, Cell, 3 (2), 127–133.

[3] Jung, H.A, Jung, H. J., Jeong, H. Y., Kwon, H. J., Kim, M. S. and Choi, J. S. (2014) Anti-adipogenic activity of the edible brown alga *Ecklonia stolonifera* and its constituent fucosterol in 3T3-L1 adipocytes, Arch. Pharm. Res., 37(6), 713–720.

[4] Jung, H. A., Jung, H. J., Jeong, H. Y., Kwon, H. J., Ali, M. Y. and Choi, J. S. (2014), Phlorotannins isolated from the edible brown alga Ecklonia stolonifera exert anti-adipogenic activity on 3T3-L1 adipocytes by downregulating C/EBPα and PPARγ, Fitoterapia, 92, 260–269.

[5] Park, Y., Lee, T., Choi, J., Sukbong, V., Lee, J., Park, J. and Jang, B. (2014) Biochemical and Biophysical Research Communications Inhibition of adipogenesis and leptin production in 3T3-L1 adipocytes by a derivative of meridianin C, Biochem. Biophys. Res. Commun., 452 (4), 1078–1083.

[6] González-Castejón, M., García-Carrasco, B., Fernández-Dacosta, R., Dávalos, A. and Rodriguez-Casado A. (2014) Reduction of adipogenesis and lipid accumulation by *Taraxacum officinale* (dandelion) extracts in 3T3L1 adipocytes: An *in vitro* study," Phyther. Res., 28, 745–752.

[7] Nagarajan, M., Maruthanayagam, V. and Sundararaman, M. (2012) A review of pharmacological and toxicological potentials of marine cyanobacterial metabolites, J. Appl. Toxicol., 32 (3), 153-185