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Annals of Medicine

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iann20

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To cite this article: Daniela Guerreiro, Carla Ferreira, Madalena Salema-Oom & Alexandre Quintas (2021) *Saccharomyces cerevisiae* as a model to study synthetic cannabinoids: the impact of using different carbon sources, Annals of Medicine, 53:sup1, S80-S81, DOI: 10.1080/07853890.2021.1897429

To link to this article: https://doi.org/10.1080/07853890.2021.1897429

Published online: 28 Sep 2021.

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DOI: 10.1080/07853890.2021.1897428

Saccharomyces cerevisiae as a model to study synthetic cannabinoids: the impact of using different carbon sources

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ABSTRACT

Introduction: Synthetic cannabinoids (SC) are potent agonists of cannabinoid receptors, that mime the psychoactive effect of Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive component of cannabis [1]. JWH-018 was the first novel psychoactive SC found in the recreational drug marketplace in 2008. A few years later, JWH-018 started to be controlled by authorities, so alternative molecules started to emerge. THJ-018 was designed to replace JWH-018, having a similar structural skeleton and also a naphthalene and pentyl chain connected *via* a middle core substructure. Eventually, THJ-018 was scheduled and alternatives emerged, such as EG-018 [2]. This practice makes almost impossible to characterise SC toxicological profiles on an acceptable time scale, mostly due to the time-consuming experiments that must be held in animal models or human cells by standard methods. The yeast *Saccharomyces cerevisiae* shares highly conserved molecular and cellular mechanisms with human cells and has been used before for synthetic cathinones [3]. The present work has studied the best carbon source (glucose or galactose) to measure the impact of synthetic cannabinoids on *S. cerevisiae* growth, aiming to develop a method able to profile synthetic cannabinoids toxicity in a short time scale. The difference between carbon sources is that in Crabtree-positive yeast strains, glucose induce a strong inhibition of mitochondrial oxidative phosphorylation [4].

Materials and methods: The effect of EG-018 was evaluated by its impact on *S. cerevisiae* BY4741 (WT) growth on minimal medium with 2% Glucose or 1% Galactose as sole carbon sources, in the presence and absence of the SC, followed at OD_{600nm}. Growth rates at each condition was fitted to a logistic equation.

Results: Figure 1 shows OD_{600max} obtained from the non-linear regression to the logistic equation in the presence of different concentrations of EG-018. While in galactose there is no effect, in 2% Glucose the results points to a growth decrease with EG-018.

Discussion and conclusions: Comparing the two carbon sources, yeast growth on glucose is more susceptible to EG-018. Recent studies points that JWH-018, a similar SC, exerts an effect on glycolytic and pentose phosphate pathway at high concentrations of glucose (personal communication). As yeast growth in galactose proceeds simultaneously *via* respiration and fermentation due to the "Crabtree effect" [4] an impact of EG-018 on glycolysis might be less effective. These results, suggest that glucose is the best carbon source to develop a yeast based toxicity sensor for SC.

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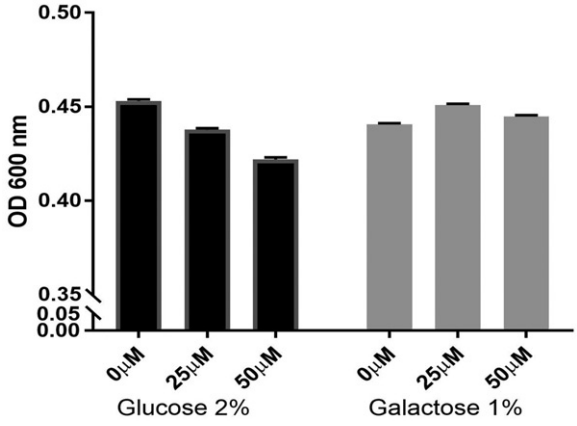

Figure 1. OD_{600max} results in S. cerevisiae BY4741 in the presence of 0, 25 and 50 µM of EG-018 in glucose and galactose.

Acknowledgements

This study was funded by Cooperativa de Ensino Superior Egas Moniz.

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DOI: 10.1080/07853890.2021.1897429

Toxicity of synthetic cannabinoids is increasing along with the regulatory measures taken for their control

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ABSTRACT

Introduction: Novel psychoactive substances (NPS) represent a rising public health threat due to the associated side effects and the lack of success on their control policies. NPS are freely sold, mostly on the internet, as legal and safe replacements of controlled drugs of abuse. Synthetic cannabinoids (SCs) represent one of the largest groups of NPS and are designed as mimetics of Δ9-tetrahydrocannabinol from cannabis [1]. After the identification of JWH-018, the first SC appearing on the market, many countries took measures to control the free circulation of these products [2]. Nevertheless, efforts to control them are rapidly contoured, since clandestine manufacturers are constantly changing their chemical structures leading to previously unknown SCs [3]. The aim of this study is to show how chemical changes on recent generations of SCs to evade the law lead to the production of more harmful compounds, with potential application in forensic context. With that purpose, the toxicity of three SCs, representatives of the first, second and third generations [4], was assessed.

Materials and methods: The SC JWH-018 was obtained as >98.5% pure powders from Lipomed AG Switzerland. SCs THJ-018 and EG-018 were bought through an internet website (www.chem.eu). THJ-018 and EG-018 were purified through HPLC/DAD and confirmed by GC/MS. Pure SCs stock solutions were prepared in 100% DMSO accordingly to the stipulated final concentrations and diluted into the culture medium prior addition to cells. The neuroblastoma human cell line SH-SY5Y was exposed, for 24 h, to several concentrations of each SC. Cell toxicity was evaluated through MTT assays.

Results: Figure 1 shows the MTT results for SH-SY5Y viability in the presence of JWH-018, THJ-018 and EG-018. SH-SY5Y cells viability does not decrease in the presence of JWH-018 at the range of concentrations used. However, when the same cell line is exposed to THJ-018 or EG-018 there is a decrease in cell viability with the increase in the concentration of these substances.

Discussion and conclusions: The results from the present study points THJ-018 and EG-018, JWH-018 analogues of 2nd and 3rd generations, as more toxic to the neuroblastoma cells than JWH-018, the first SC found on the street market. These results suggest that emerging modified SCs, to avoid the law, are becoming more toxic and dangerous. Given the emergence of this situation, it is time to rethink current legislation to prevent rise on public health issues derived from consumption of molecules of unknown toxicological profile.

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Acknowledgements

The authors acknowledge funding from Egas Moniz – Cooperativa de Ensino, CRL.