Synthetic cannabinoids consumption are a risk factor on the onset of Parkinson's Disease

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INTRODUCTION

Parkinson's Disease (PD) is the second most prevalent neurodegenerative disease and is related to the protein α-synuclein (αsyn). Phytocannabinoids are used legally or illegally by PD patients worldwide. In general, the effects of phytocannabinoids on PD appear to be protective either by binding to the CB1 receptor or CB2[1,2]. The binding of Δ^9-THC to CB1 restores membrane potential, decreases ROS, increases CB1 protein level, which will increase the amount of synaptic vesicles inhibition[3–5]. Moreover, after a decrease of the mitochondrial membrane potential by MPP+ exposition, the activation of CB2 by cannabidiol might be neuroprotective by inhibiting the apoptosis[6]. Synthetic cannabinoids are most consumed class of Novel Psychoactive Substances and are sold as safe and legal alternatives to cannabis[7]. Despite their increasing use, there is a lack of knowledge on its toxic effects. In fact, some of the substances seem to have stronger toxicological effects when compared to their legal counterpart[8]. Toxicological assays are paramount to know how harmful these new substances are, helping increase public awareness since several hospitalization cases have been reported due to consumption. Yeast Saccharomyces cerevisiae was chosen as a model to understand the relationship between synthetic cannabinoids consumption and the increased risk for the onset of sporadic forms of PD. This organism has been proven an invaluable model to study the fundamental molecular mechanisms involved in several neurodegenerative human diseases[9]. The impact of NPS consumption in PD is yet to be revealed since it is a recent trend. Notewithstanding, the data presented suggests that hese substances contributes to PD onset.

Growth curves analysis

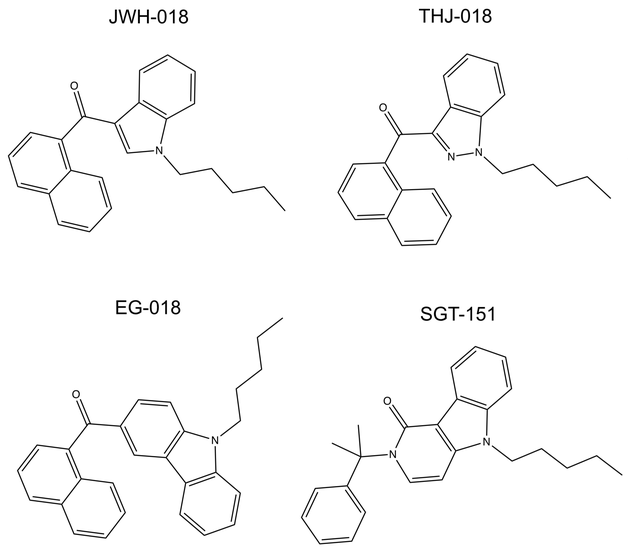

Fig. 1 – **Synthetic cannabinoids.** Selected synthetic cannabinoids were JWH-018 [1-naphthalenyl(1-pentyl-1H-indol-3-yl)-methanone], THJ-018 [1-naphthalenyl(1-pentyl-1H-indazol-3-yl)-methanone], EG-018 [naphthalen-1-yl(9-pentyl-9H-carbazol-3-yl)methanone] and SGT-151 [2,5-dihydro-2-(1-methyl-1-phenylethyl)-5-pentyl-1H-pyrido[4,3-b]indol-1-one]. All synthetic cannabinoids were purified from herbalblends bought from the internet or smartshops by HPLC/DAD and confirmed by GC/MS and NMR. Stock solutions of this compound were prepared in 100% DMSO in different concentrations and they was tested in yeast cultures at 0,4% of DMSO.

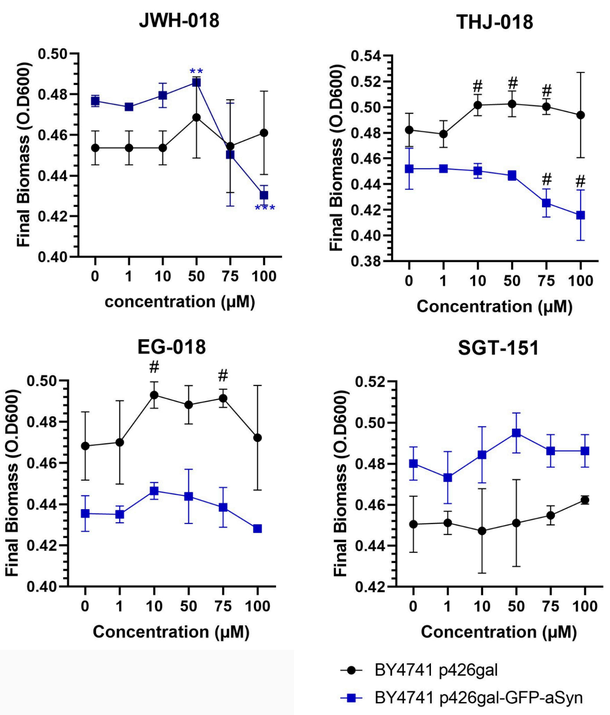

Fig. 2 – **Final biomass results of yeast growth in the presence of the synthetic cannabinoids (#p<0,1, **p<0,02; ***p<0,01).** BY4741 WT and BY4741 expressing expressing αsyn-GFP were growth in the presence of synthetic cannabinoids and its growth curves were monitored. To further understand differences between yeast growth rates in the presence and absence of synthetic cannabinoids, the growth curves were fitted to a logistic equation, which originates final biomass, growth rate and the doubling time. The kinetic growth result showed that SGT-151 and EG-018 do not appear to be toxic to yeast cells that express α-syn. Moreover, JWH-018 increases cell viability at lower doses. However, at high doses, JWH-018 is toxic. THJ-018 also seems to affect the cells final biomass.

The impact of JWH-018 in α-syn

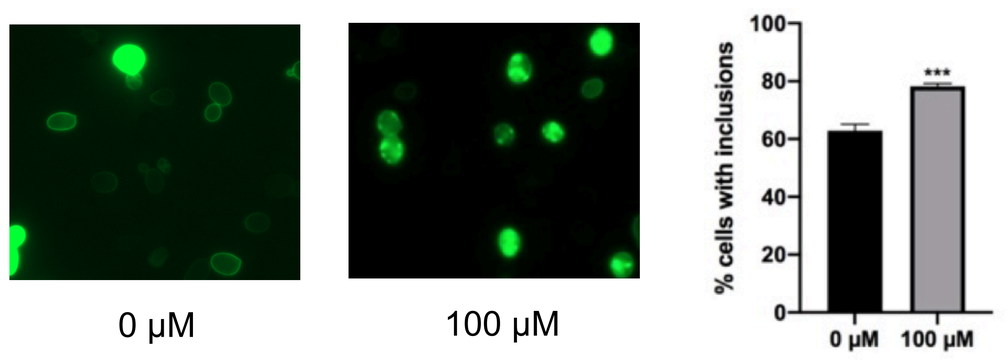

Fig. 3 – **Intracellular localization of the WT αSyn-GFP and percentage of BY4741 WT yeast cells containing αSyn-GFP inclusions, assessed by fluorescence microscopy after 14 hours of αSyn-GFP expression induction (*** p<0.001).** The results presented in figure 1A showed that in the presence of 100 μM, there were more cells with inclusion than in the absence of compound. These inclusions are toxic for cells.

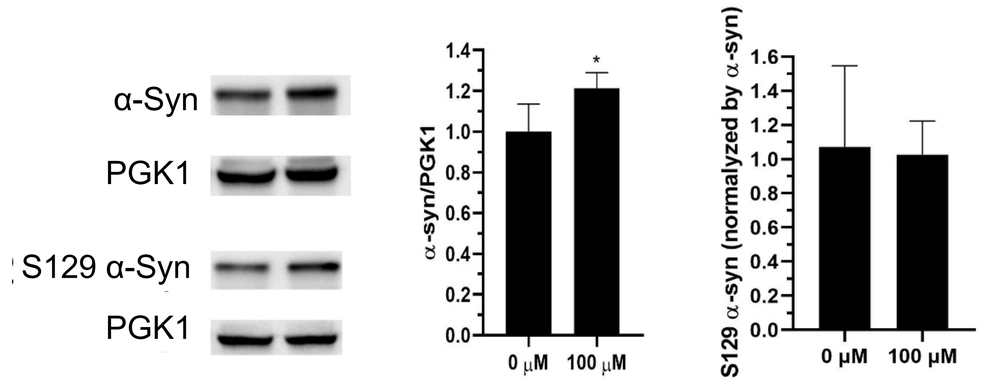

Fig. 4 – **Total αSyn and S129 αSyn in the presence and absence of JWH-018 *p<0,05.** WT αSyn protein levels in yeast cells assessed by western blot analysis of total protein extracts at 14h of αSyn-GFP expression. Densitometric analysis of the immunodetection of αSyn relative to the intensity obtained for PGK1, used as loading control, presented in arbitrary units (a.u.) *p<0.05). S129A αSyn protein levels in yeast cells assessed was also studied, once phosphorylation modulates clearance of αSyn Inclusions[10]. S129-αSyn levels were determined by doing the ratio between both values: (pS129/PGK1)/(αSyn/PGK1) and normalised to the control. The results presented in figure, show that there is more protein in the presence of the compound, which can be the cause that increases the a-syn inclusions. Otherwise, the a-syn phosphorylated did not show differences, meaning that the postradutional modification is not involved in the molecular mechanism underlying the increase of αSyn.

CONCLUSIONS

The results in this study showed that synthetic cannabinoids are toxic to yeast cells and can have an impact on αsyn, since yeast cells expressing a-syn and in the presence of synthetic cannabinoids decrease its final biomass. Moreover, BY4741 WT yeast cells in the presence of 100 μM of JWH-018 containing more αSyn-GFP inclusions. These inclusions were justified by the increase of αsyn protein in the presence of JWH-018. These observation might be explained by a dysregulation, in the presence of the compound, of a cellular mechanism responsible for protein degradation. As far as we know, this is the first approach to study the molecular mechanisms of Parkinson's Disease induced by synthetic cannabinoids.

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