

CannaLatan - International Society for Neurochemistry School “Investigating cannabinoid function in the nervous system: mechanism of action and biomedical implications”



Neuroprotective property of *Cannabis* extracts and cannabinoids: *in vitro* studies

Laboratorio de Mecanismos de Neurodegeneración y Neuroprotección.
Departamento de Neuroquímica, IIBCE
Montevideo, Uruguay



Ministerio
de Educación
y Cultura



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Junio, 2022

OVERVIEW

- Neuroprotection
- Phytocannabinoids as neuroprotective agents
- Our Results

Isolated phytocannabinoids

Combined phytocannabinoids

Cannabis-based extracts:

Pharmaceutical products-with high content of CBD

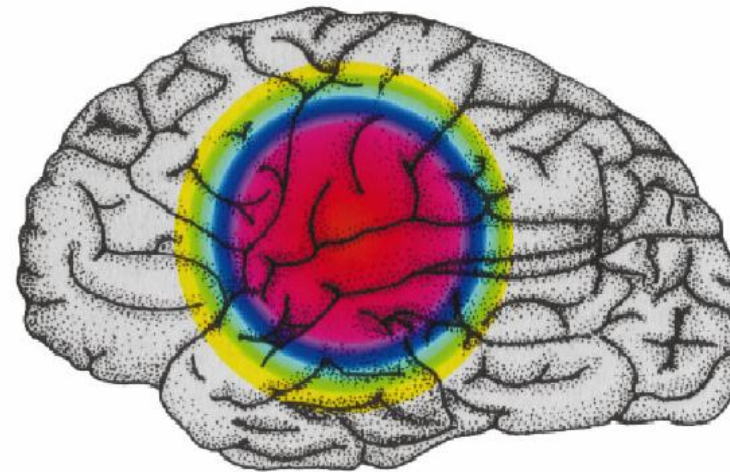
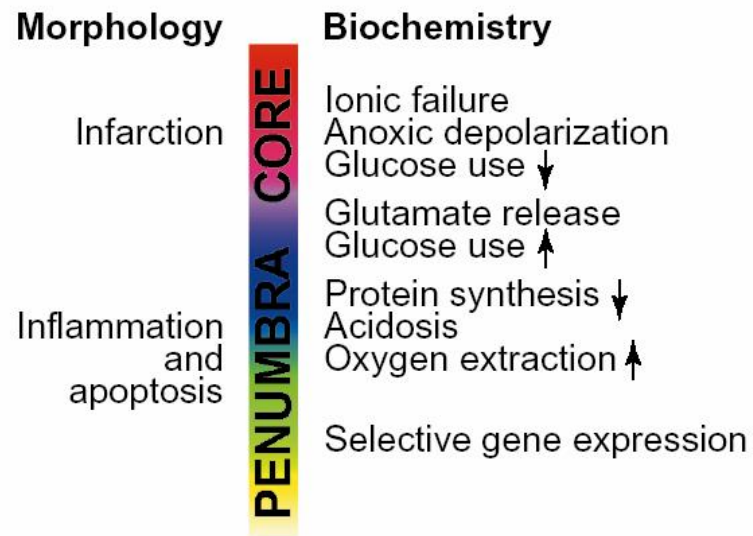
Whole-plant cannabis extracts



❖ Neuroprotection involves prevention of neuronal death

Ischemia/Stroke

In the 1960s the term "neuroprotection" was applied to the protection of the brain during high-risk neurosurgical that required the interruption of blood circulation to the brain

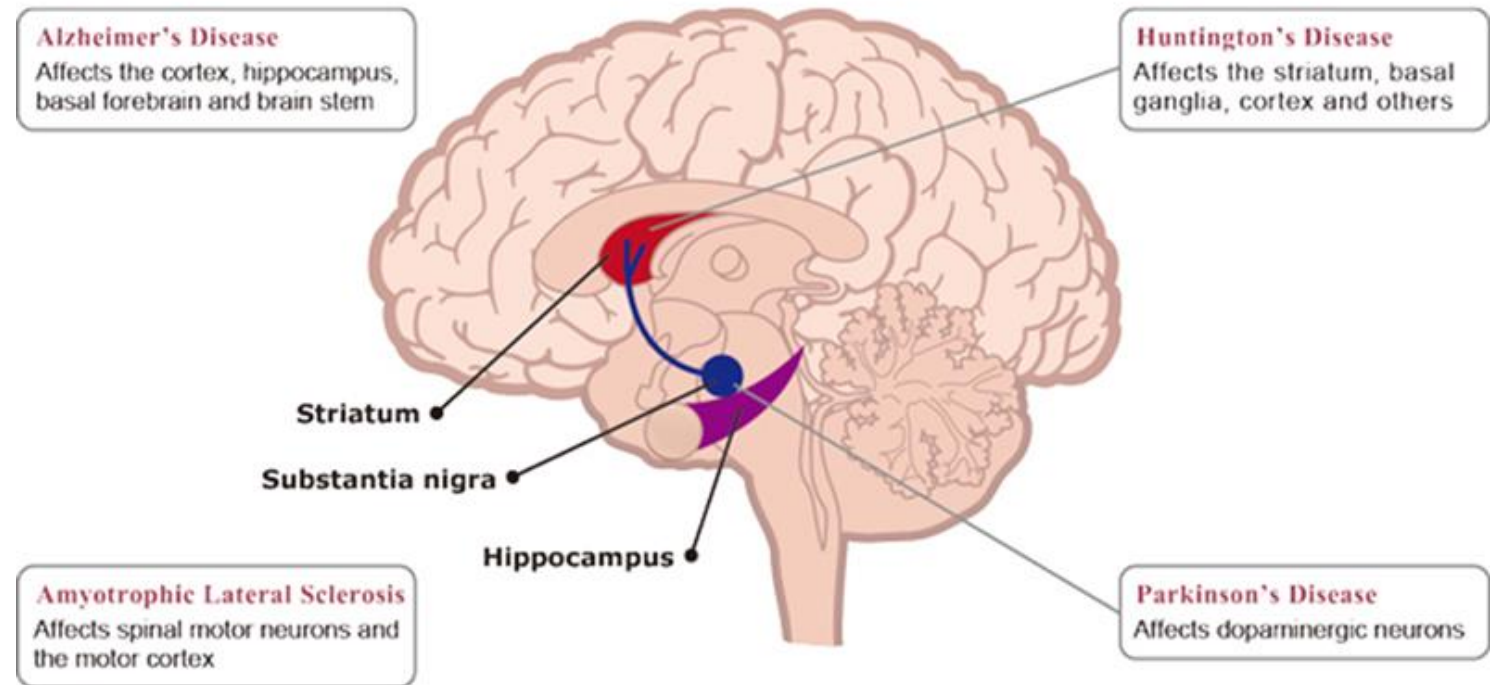


trends in Neurosciences

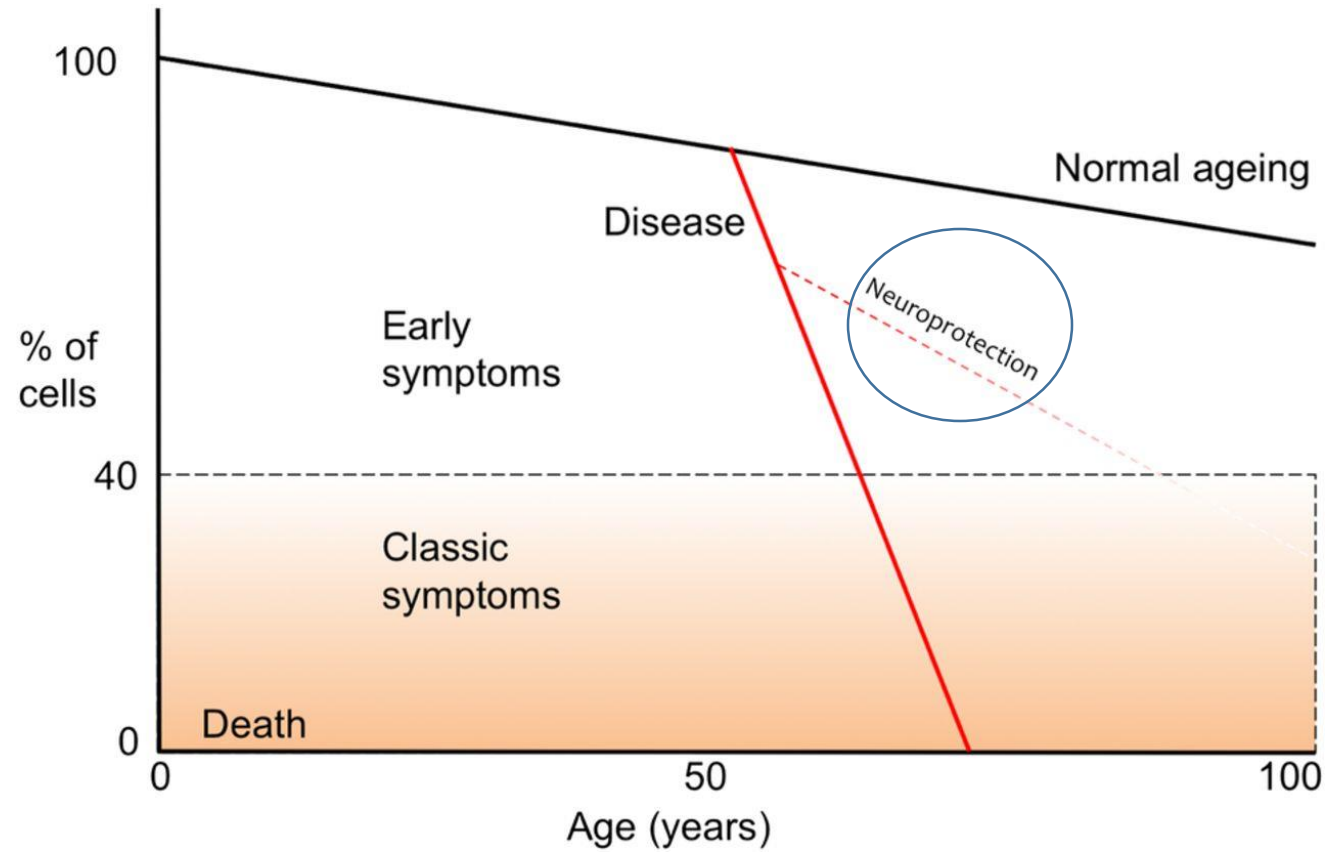
Neurodegenerative diseases

Are characterized by progressive and irreversible neurons loss from specific regions of the brain

- ✓ Are the leading causes of death in developed countries.
- ✓ Epidemiological data indicate that the incidence worldwide has increased significantly in the last 30 years.
- ✓ Demand high economic and emotional costs for the patient and their close environment.
- ✓ The therapies available are symptomatic and do not stop or delay the degenerative process.



Neuroprotective therapies in neurodegenerative diseases



Neuropsychiatric disorders

schizophrenia, bipolar disorder, and major depression

- ✓ Accumulating data from postmortem and brain imaging studies revealed morphological changes in the brain of patients with these mental disorders, such as ventricle enlargement, volumetric reduction, loss of neurons and glial cells in particular cortical and limbic brain regions (Hunsberger et al 2009, Wee et al 2016).

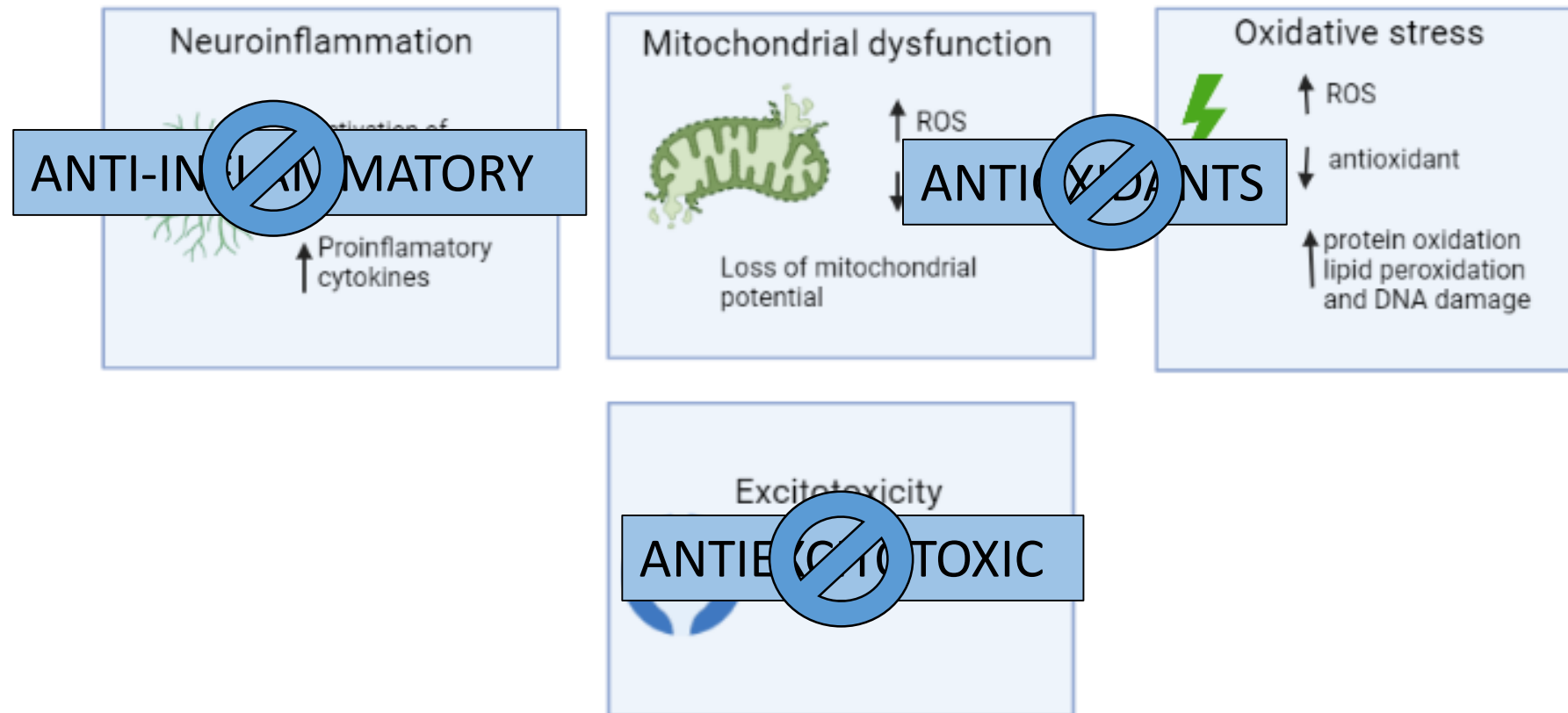


Table 1
Neuroprotective properties of agents that may be used for mental health.

	MRI evidence of prevention of brain volumetric changes	Neuroprotective in cell cultures or animal models
Lithium	Yes	H ₂ O ₂ treated cortex cells
Clozapine		LPS treated neuron-glia cells
Aripirazole		Glutamate treated neurons
Olanzapine		1. Nutrient deprived cells. 2. Mouse cerebral ischemia.
Paliperidone		H ₂ O ₂ treated neurons
Quetiapine		INF-γ treated microglia
Ziprazadone		INF-γ treated microglia
Perospirone		INF-γ treated microglia
Antidepressants		LPS treated neuronal stem cells
Mincycline		1. Glutamate treated neurons. 2. Mouse cerebral ischemia.
Statins		
Aspirin		Rodent cerebral ischemia
Omega-3 PUFA		
Erythropoietin		Trimethyltin treated neurons
Melatonin	Yes	Rodent brain; Human brain cells.
Leptin		↓MDD, ↓Alzheimer degen

- ✓ Studies support the notion that psychotropic agents used to treat the major psychiatric disorders are associated with significant neurotrophic/neuroprotective effects.
- ✓ Has opened a novel avenue of exploring the causes and healing of neuropsychiatric diseases

- Although the etiology of neurological diseases is different, they all share similar biochemical events that lead to cell malfunction and subsequent death.



- ✓ To date, there is no drug in the clinic that is sufficiently effective and safe to be used as a neuroprotective in acute or chronic neurological diseases.

Neuroprotective therapies

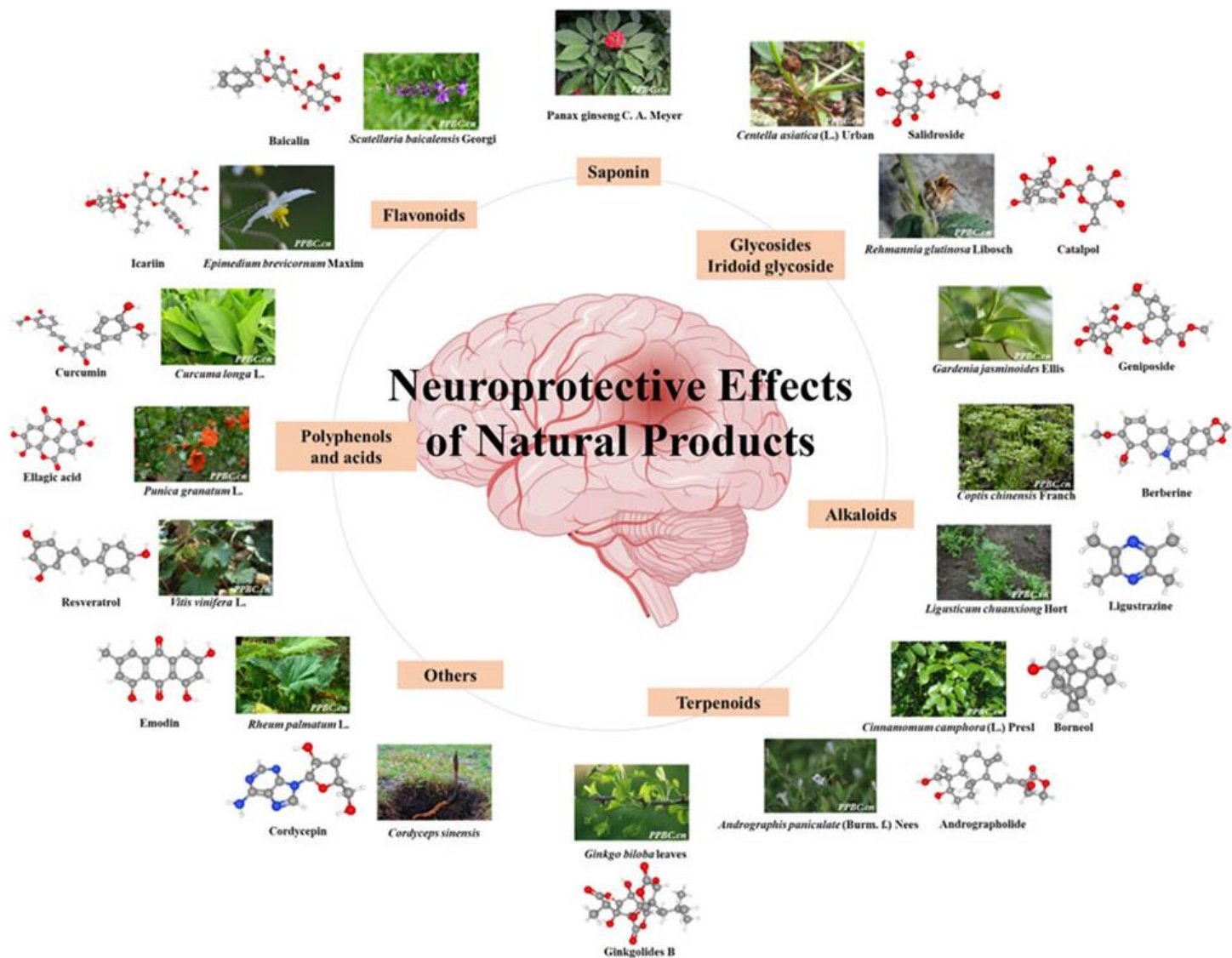
- ✓ Which of these different altered cellular functions should be prioritized as a therapeutic target?

POLY-PHARMACOLOGICAL STRATEGY

- broad spectrum agents capable of limiting simultaneously some of the cytotoxic mechanisms.
- combination of more selective agents

Natural products (NPs) from plants as Neuroprotective agents

The term “natural product” = “secondary metabolite”



Synthesis of natural products is a result of enzymatic interactions. Therefore, their biological activity involves protein binding making them effective drug candidates.

Their use in traditional medicine may provide insights regarding efficacy and safety. Ethnobotany, has always served as a starting point for drug discovery programs from herbs

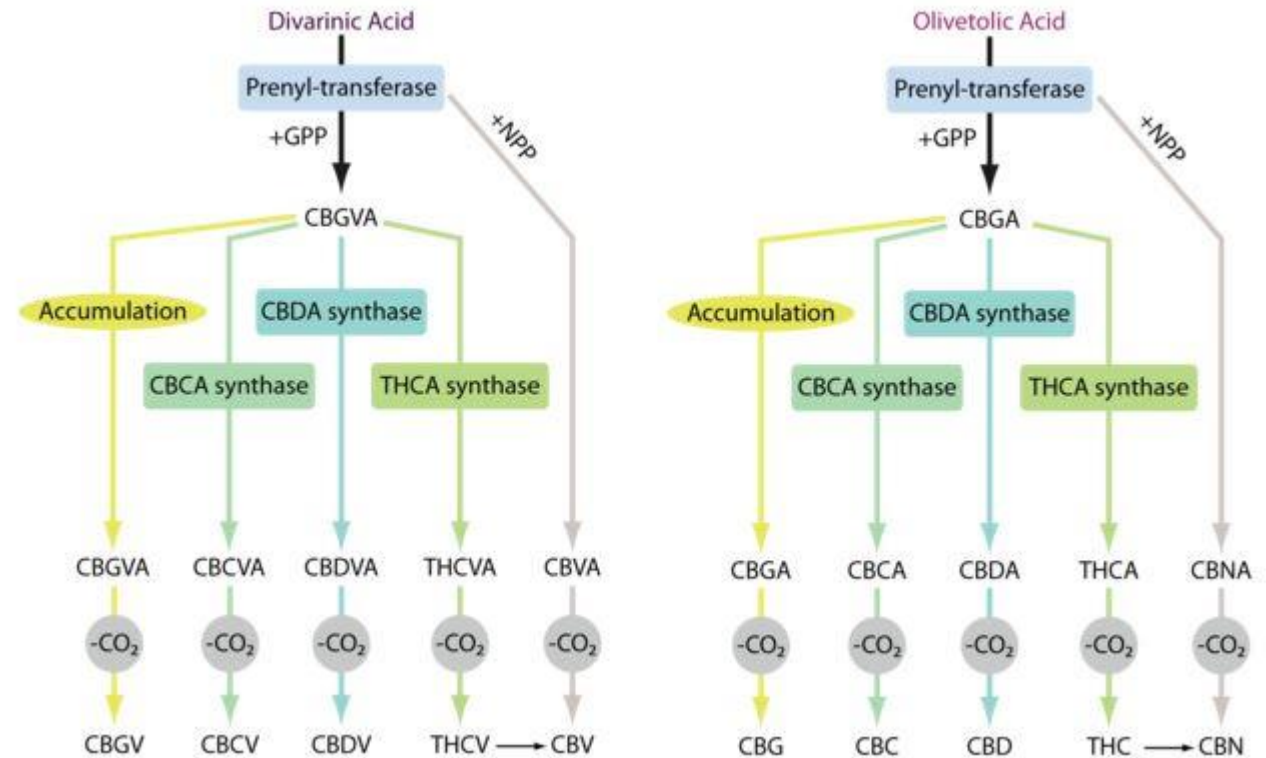
Cannabis sativa and Phytocannabinoids

Cannabis sativa L. has a long history as a medicinal plant and was fundamental in the discovery of the endocannabinoid system.

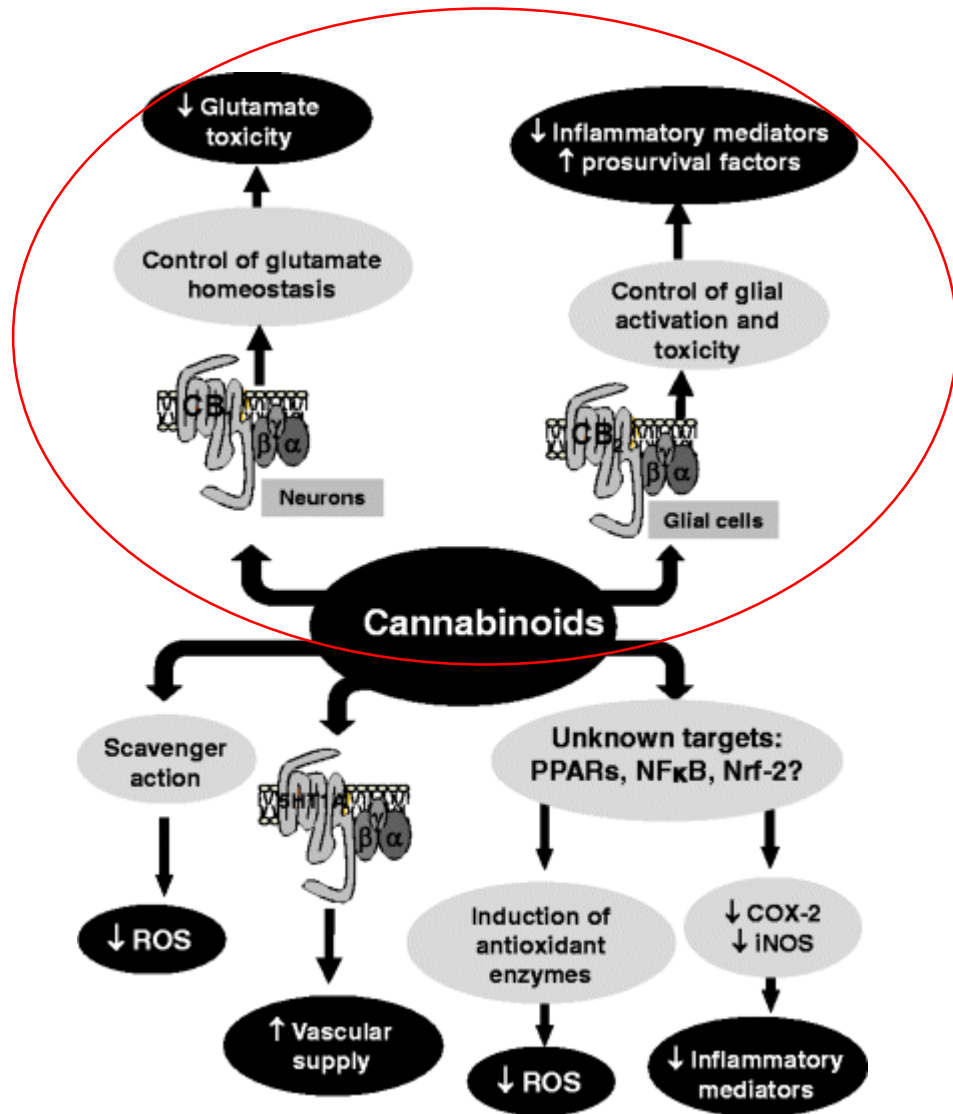
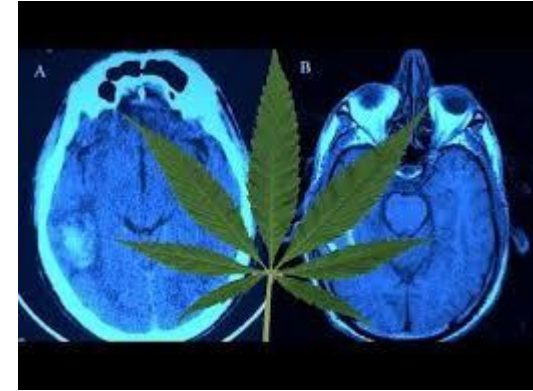
Phytocannabinoids was originally defined in a phytochemical context to refer to a structurally homogenous class of meroterpenoids typical of cannabis (*Cannabis sativa* L.)

This chemical classification is broadly based on their derivation from a common C21 precursor (cannabigerolic acid (CBGA), or its C19 analog (cannabigerovarinic acid (CBGVA))

Phytocannabinoids as any plant-derived natural product capable of either directly interacting with endocannabinoid system



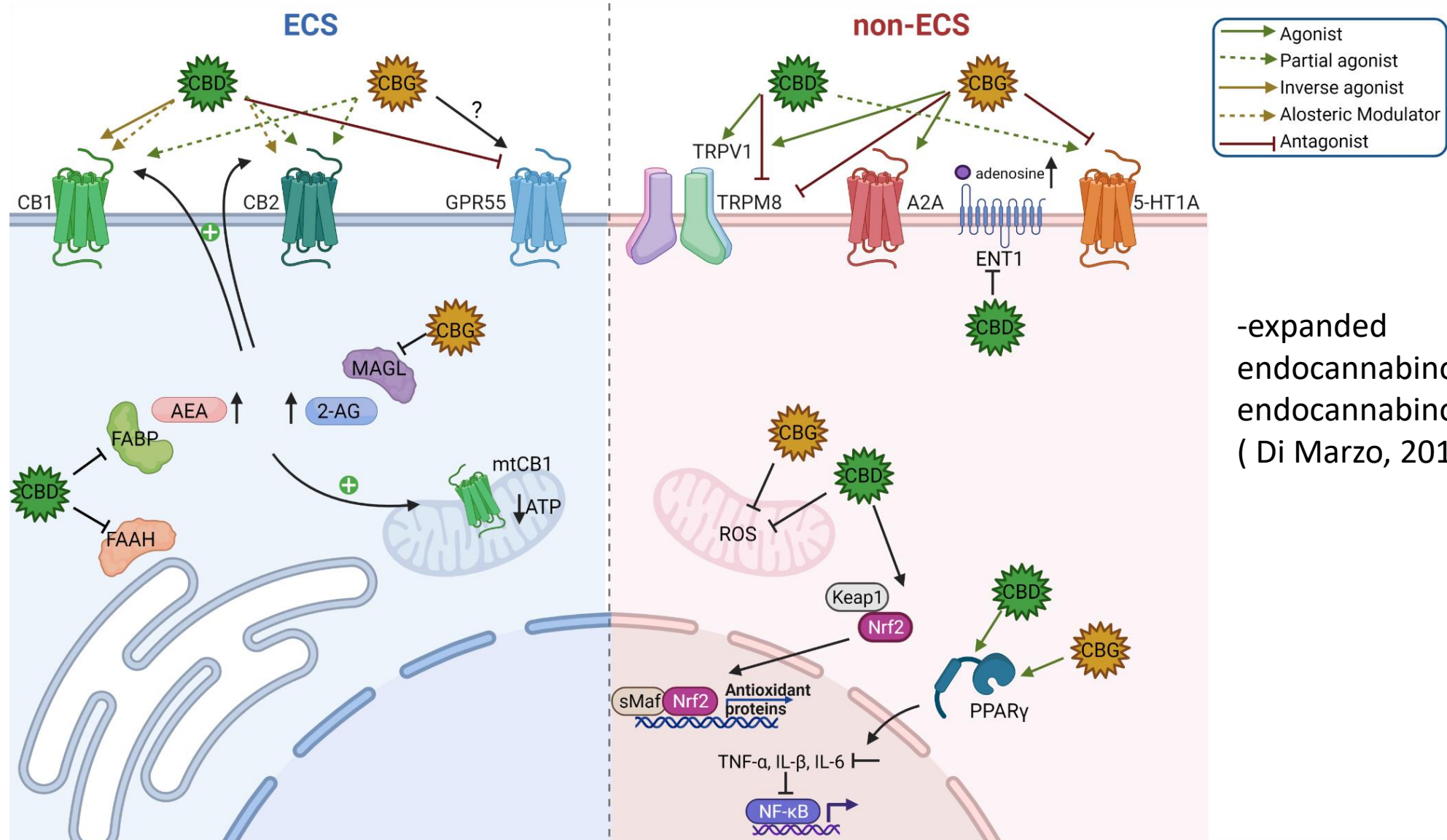
Phytocannabinoids as neuroprotective agents



Its potential as NEUROPROTECTIVE AGENTS is based on:

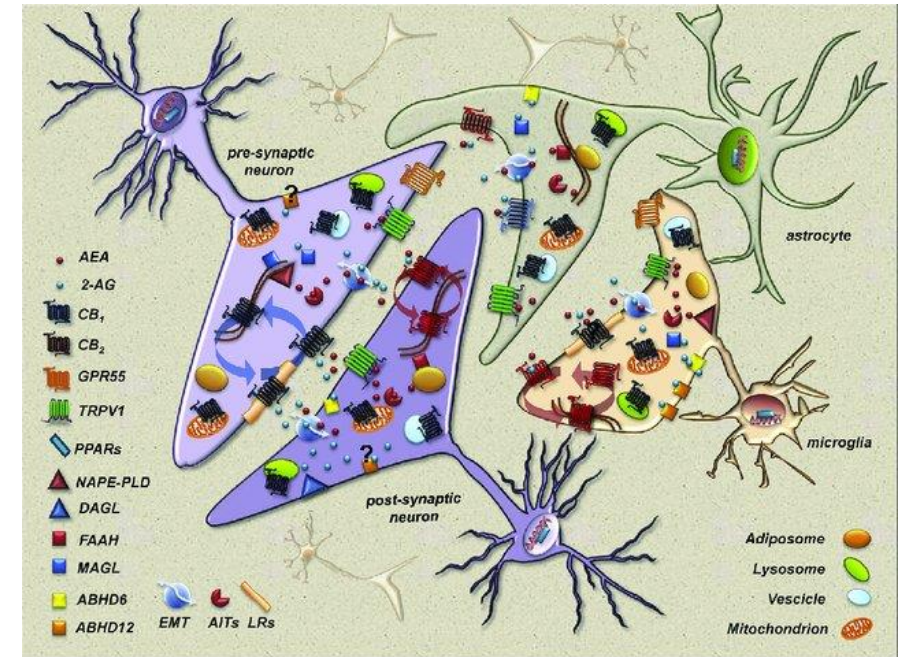
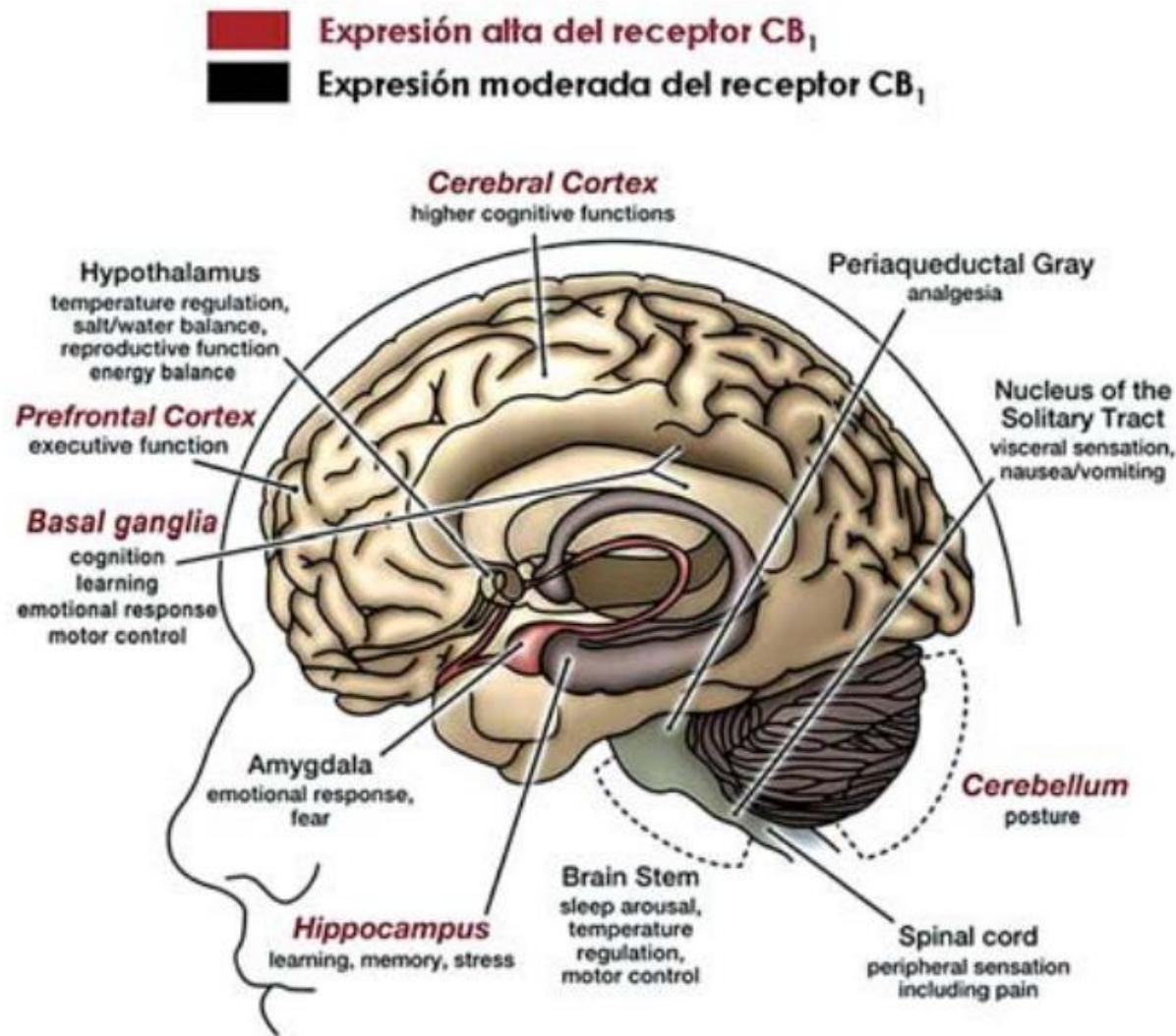
1. Wide spectrum of action: within the endocannabinoid system (ECS), and also outside this neuromodulatory system.

Phytocannabinoids as neuroprotective agents



-expanded endocannabinoid system-endocannabinoidome. (Di Marzo, 2019)

2. Location and distribution of those possible targets for action

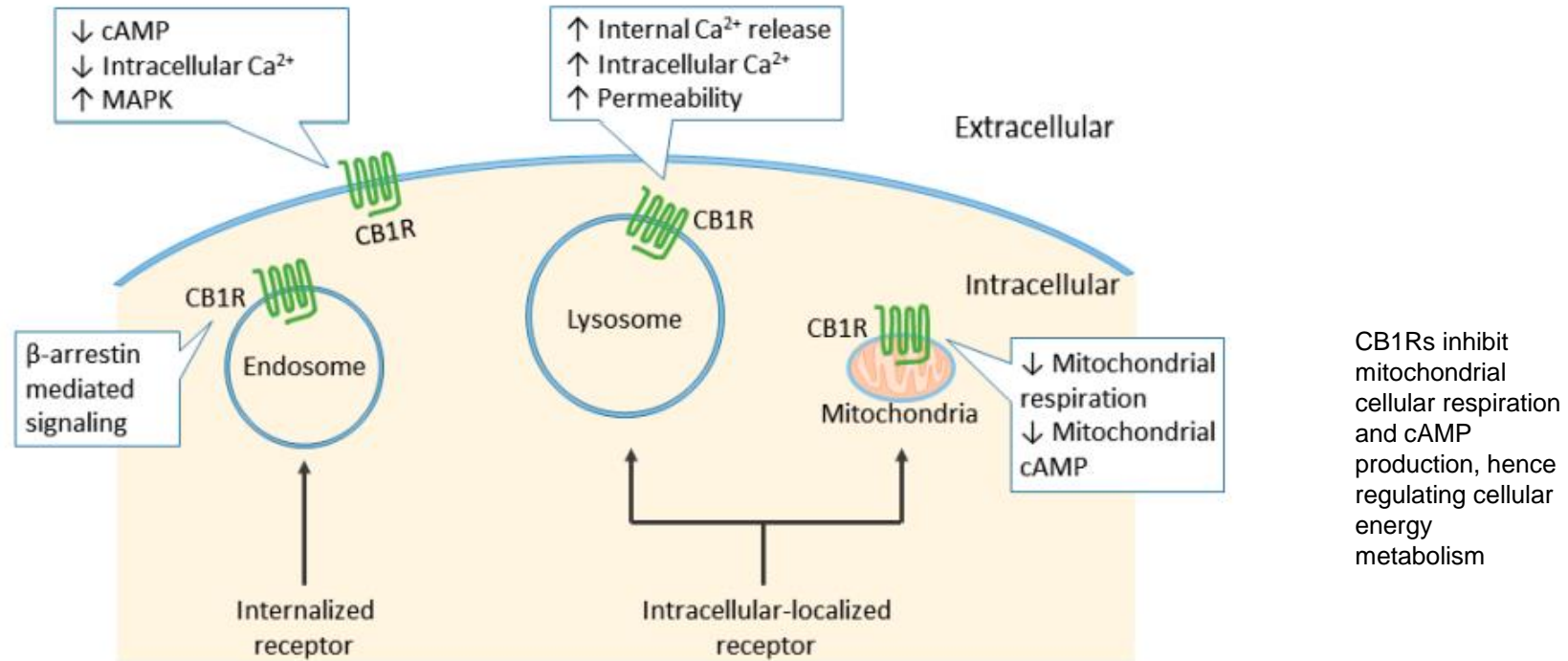


CB1Rs are found predominantly in the CNS on presynaptic axon terminals.

CB2R are present mainly in glial cells (astrocytes and microglia). High expression of CB2R mRNA was also observed in neurons, with reports showing most neuronal CB2R localization post-synaptically.

In contrast to the constitutive expression of CB1R, CB2Rs are strongly induced following trauma or pathology

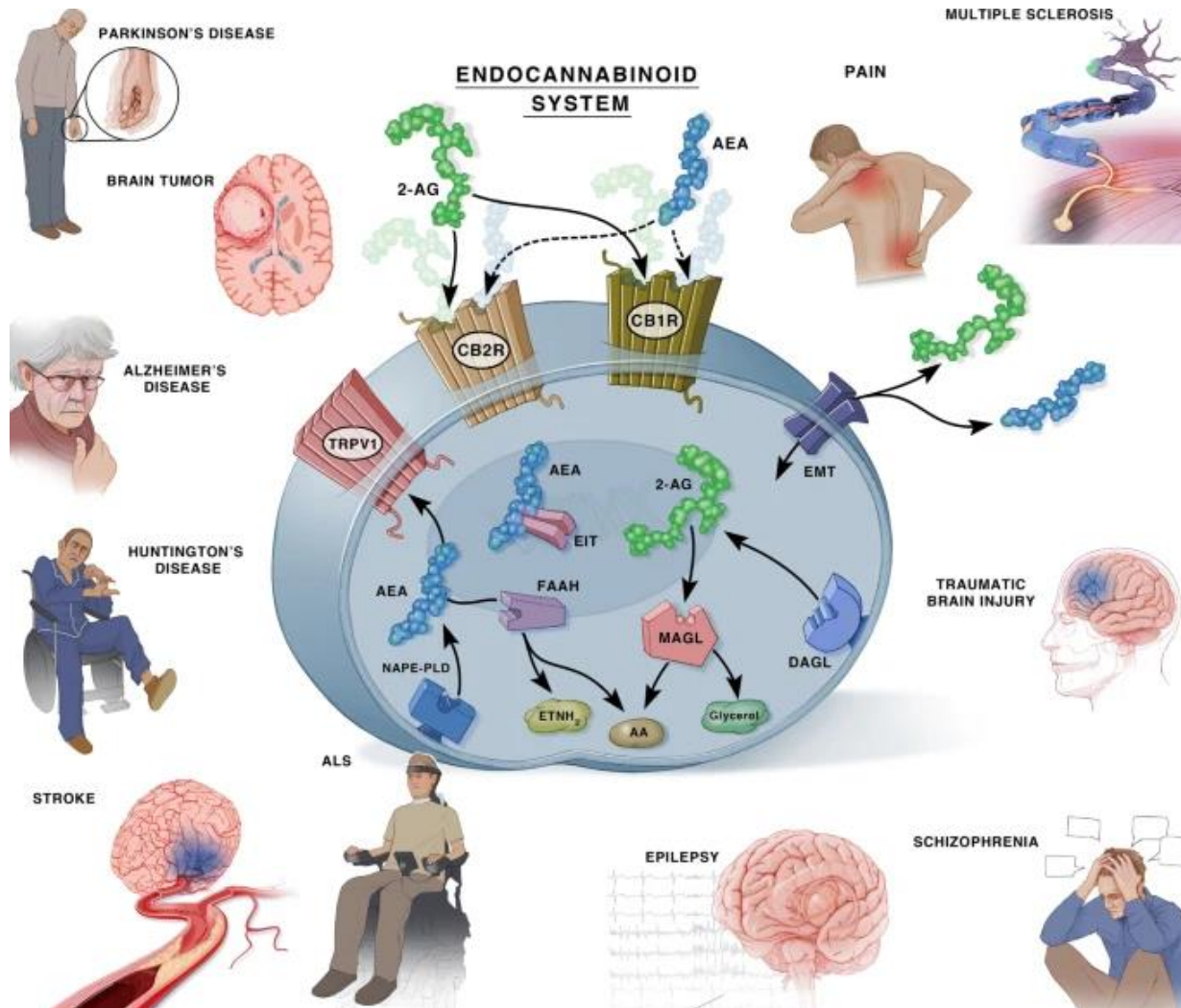
CB1-Rs: localización intracelular



Zou and Kumar, 2018

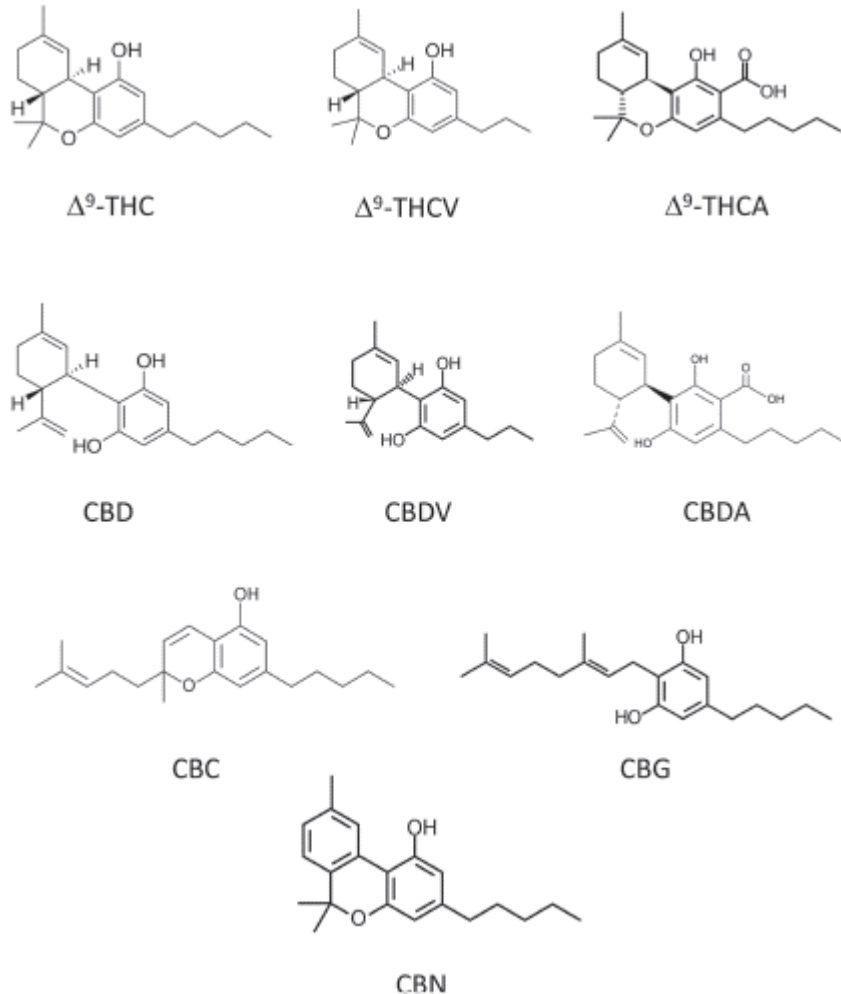
The typical distribution of CB1-R is in the plasma membrane. However, there is intracellular localization, the functions that these receptors control, eg. by internalization of receptors, or in mitochondria (Piomelli, 2014 Vallee et al., 2014) Possible connection with the neuroprotective actions of cannabinoids.

3. Evidence suggests that certain ECS components are deregulated in various neuropathologies (Cooray et al, 2020)



Potential of **phytocannabinoids** to act as new therapeutic agents in CNS disorders

Reports demonstrating the therapeutic potential of phytocannabinoids as neuroprotective agents (Stone et al., 2020).



Derivatives of CBG, known as VCE-003 or VCE-002.3 (Diaz-Alonso et al., 2016)

TABLE 3 Summary of the conditions where emerging cannabinoids have been studied

	Cannabigerol (CBG)/derivatives	Cannabidivarin (CBDV)	Cannabichromene (CBC)	Cannabinol (CBN)	Cannabidiolic acid (CBDA)	Δ^9 -THCV	Δ^9 -THCA
Huntington's	✓	-	-	✓	X	-	✓ PPAR γ ^a
Multiple sclerosis	✓	-	-	-	-	-	-
Autoimmune encephalomyelitis	✓ PPAR γ /CB $_2$ ^a	-	-	-	-	-	-
Parkinson's	✓ PPAR γ ^a	-	-	-	-	✓	✓
Neuroinflammation / neuroprotection	✓	✓	✓	✓	-	✓	✓
Epilepsy/seizure	x	✓ TRPV1 ^a	✓	-	-	✓	-
Amyotrophic lateral sclerosis (ALS)	✓	-	-	✓	-	-	-
Oxidative stress	-	-	-	✓	-	-	-
Rett syndrome	-	✓	-	-	-	-	-
Alzheimer's disease	✓	✓	✓	-	-	-	-

Note. A tick or cross represents whether a cannabinoid showed efficacy in a condition or not. A dash means that a cannabinoid has yet to be studied in a condition.
^aSome of the compounds neuroprotective effects were mediated by this receptor, but no other receptors were probed.

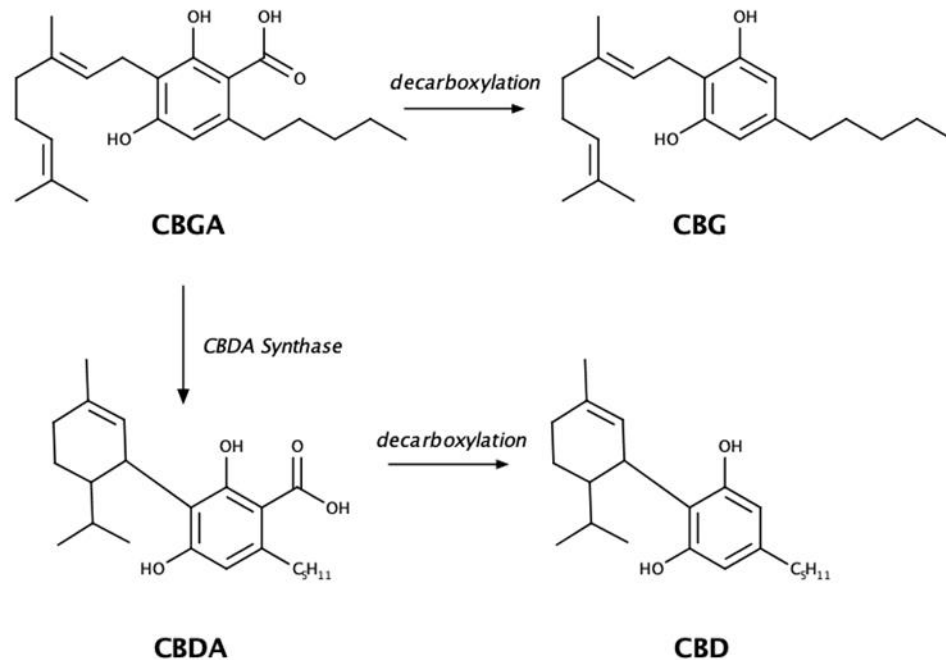
Further studies are required to investigate the full neuroprotective potential of these compounds particularly the mechanisms underlying their protective effects, as well as exploring whether their combinations may enhance their capabilities as neuroprotectors

Structured of some of the minor phytocannabinoids with cannabidiol (CBD) and tetrahydrocannabinol (Δ^9 -THC) included for reference: Δ^9 -tetrahydrocannabinolic acid (Δ^9 -THCA), Δ^9 -tetrahydrocannabinolic (Δ^9 -THCV), cannabidivarin (CBDV), cannabidiolic acid (CBDA), cannabichromene (CBC), cannabigerol (CBG), and cannabinol (CBN)



A Comparative In Vitro Study of the Neuroprotective Effect Induced by Cannabidiol, Cannabigerol, and Their Respective Acid Forms: Relevance of the 5-HT_{1A} Receptors

Carolina Echeverry¹ · Giselle Prunell¹ · Camila Narbondo¹ · Verónica Sánchez de Medina² · Xavier Nadal³ · Miguel Reyes-Parada^{4,5} · Cecilia Scorza⁶

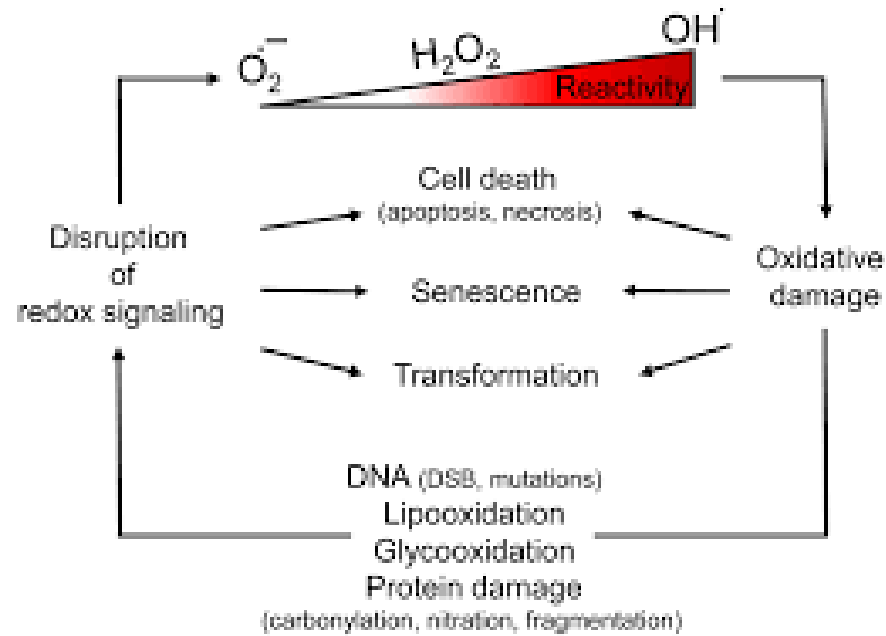


AIM

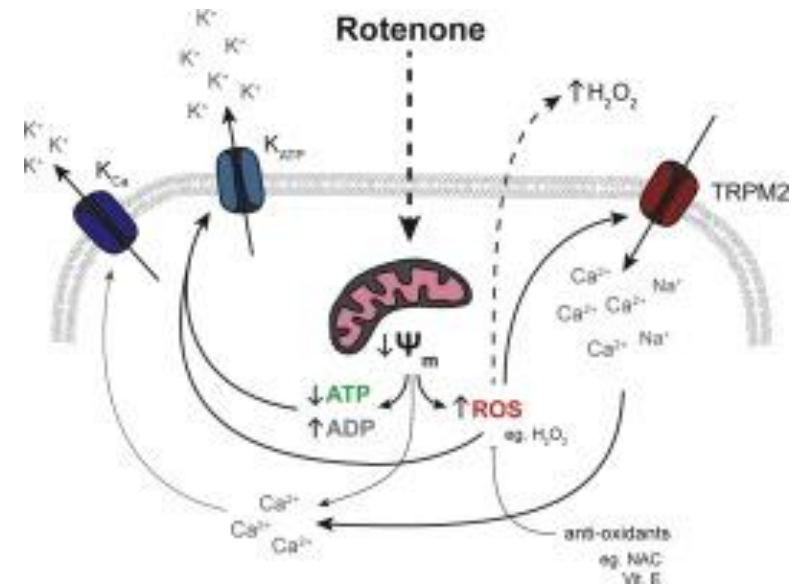
- ✓ The ability of CBD, CBG, CBDA and CBGA to attenuate the neurotoxicity induced by two insults involving oxidative stress (hydrogen peroxide, H₂O₂) and mitochondrial dysfunction (rotenone) was evaluated in neural cell cultures.
- ✓ The involvement of CB-1 and CB-2, PPAR γ or 5-HT_{1A} receptors was investigated.

Neurotoxic insults:

Oxidative stress: Hydrogen Peroxide

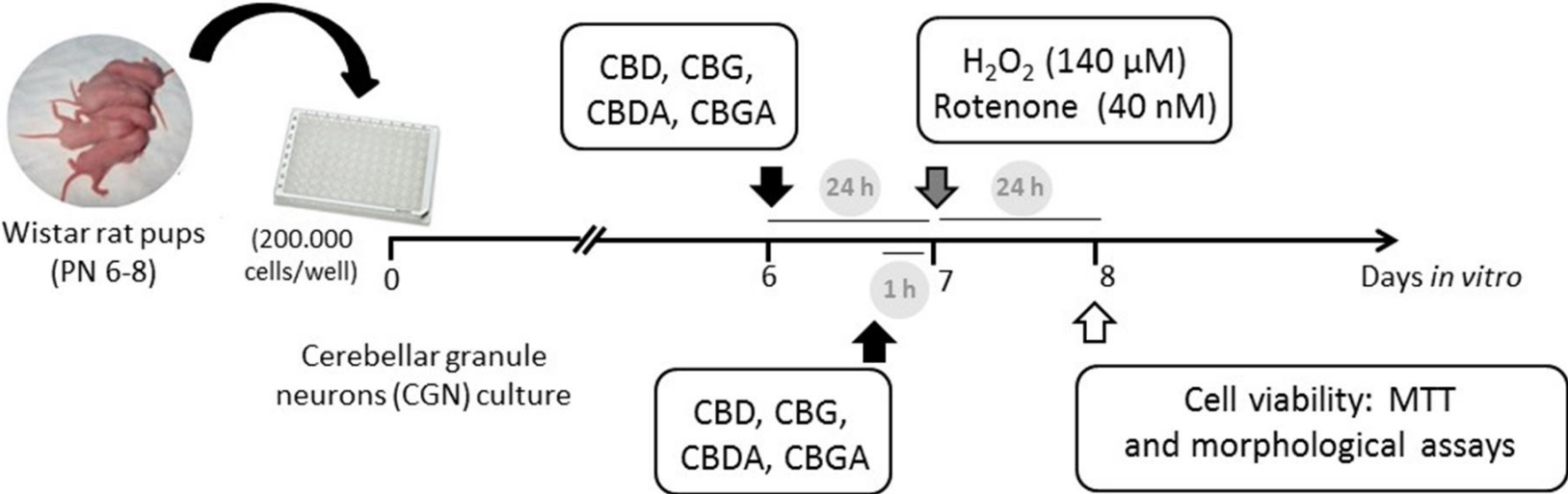
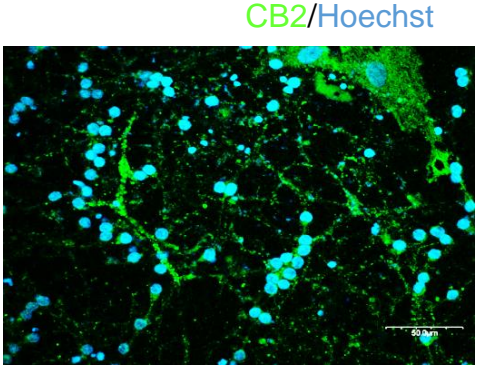
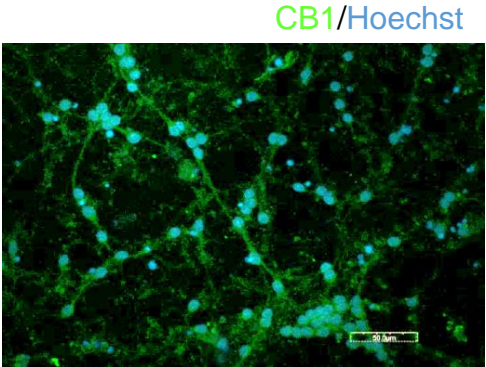
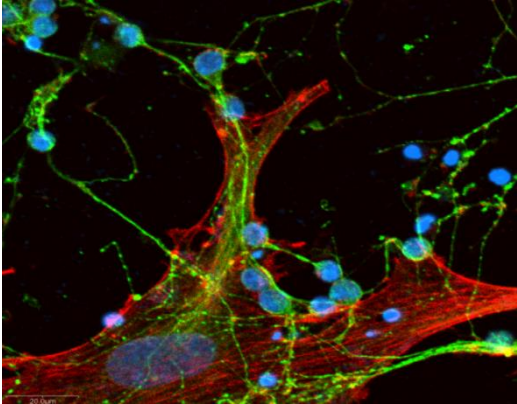
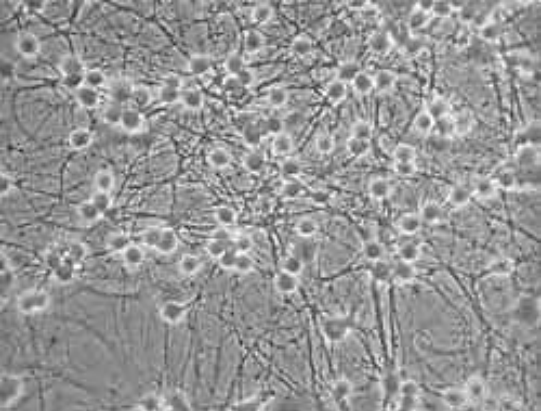


Mitochondrial dysfunction: Rotenone



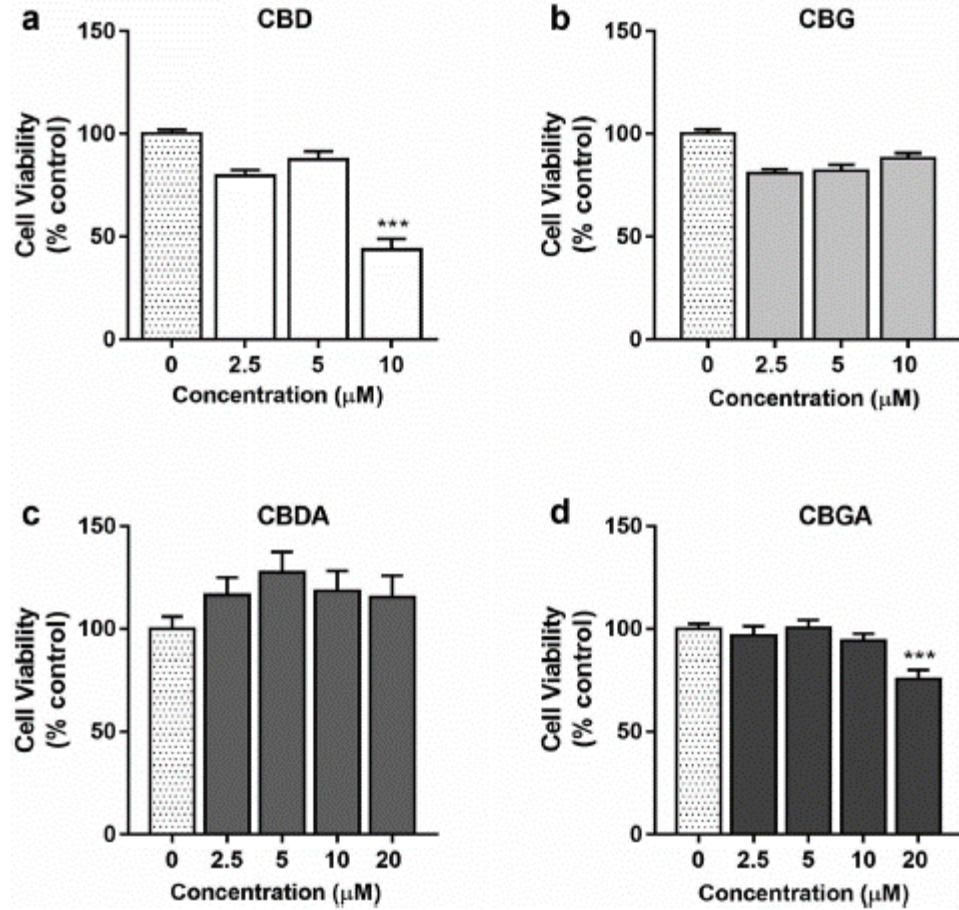
Experimental design:

- Primary culture of cerebellar granule neurons

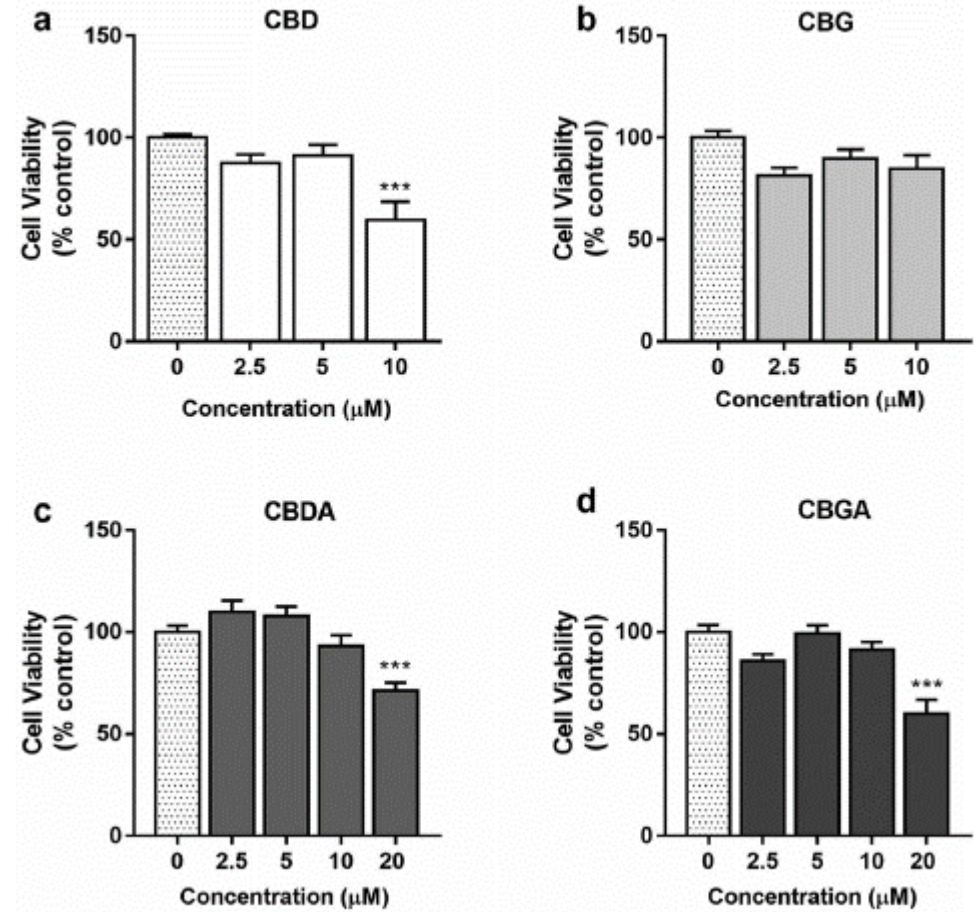


Neurotoxicity assays of CBD, CBG, CBDA and CBGA

Cannabinoids neurotoxicity (24 h)

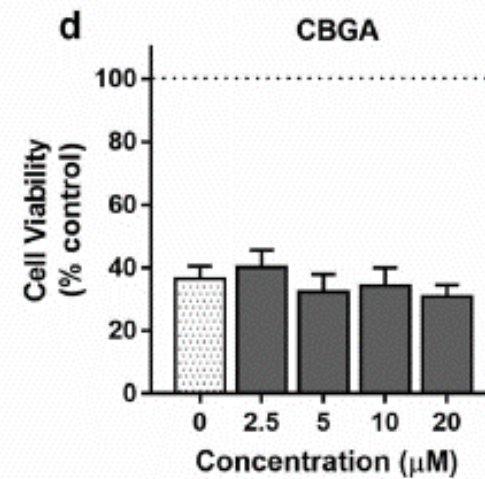
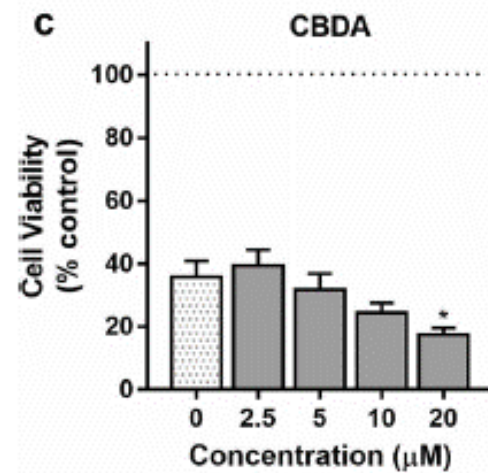
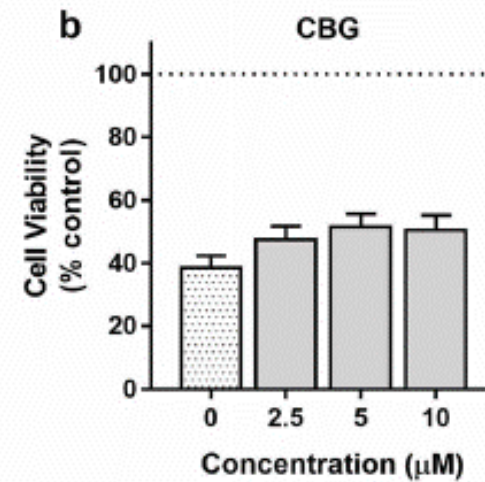
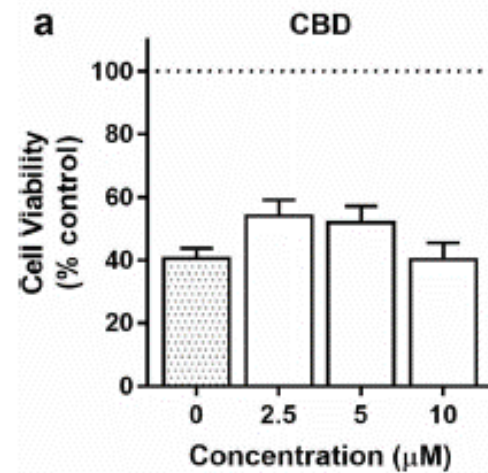


Cannabinoids neurotoxicity (48 h)



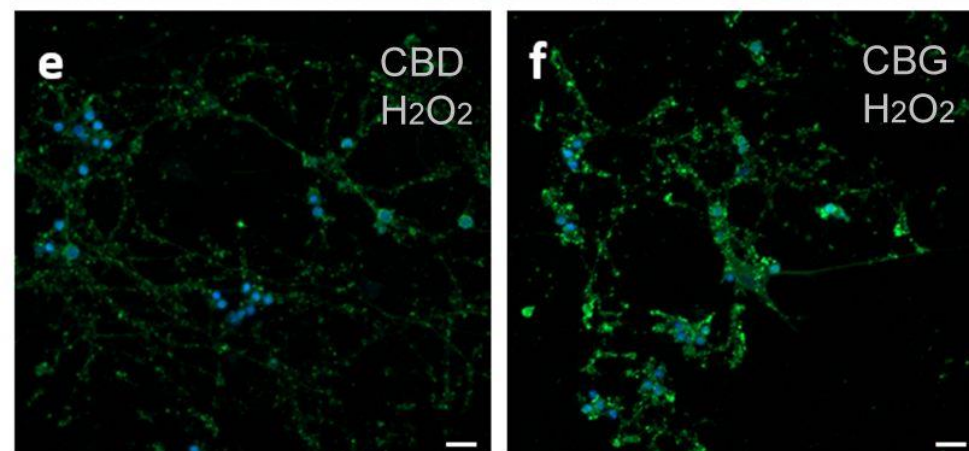
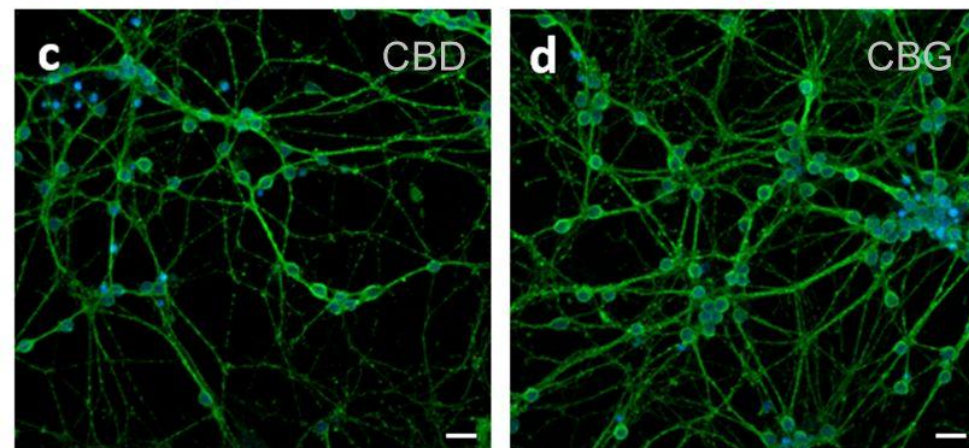
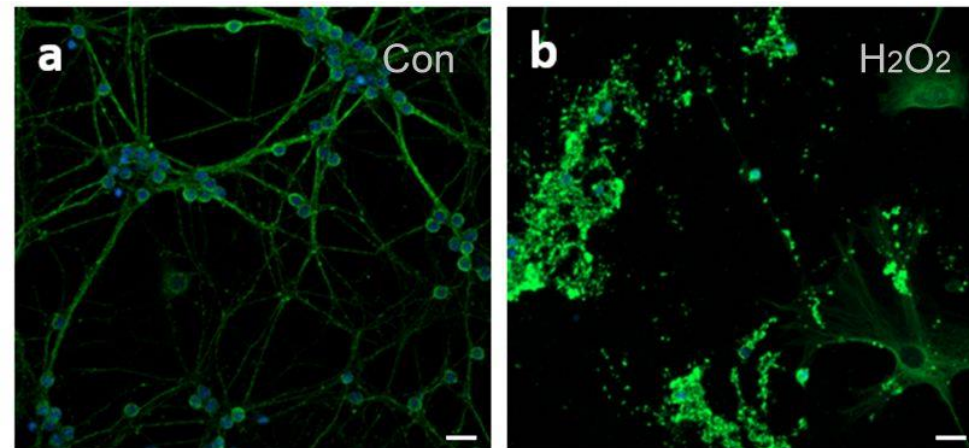
Neuroprotection assay against H₂O₂

Cannabinoids neuroprotection - H₂O₂ (24 h)

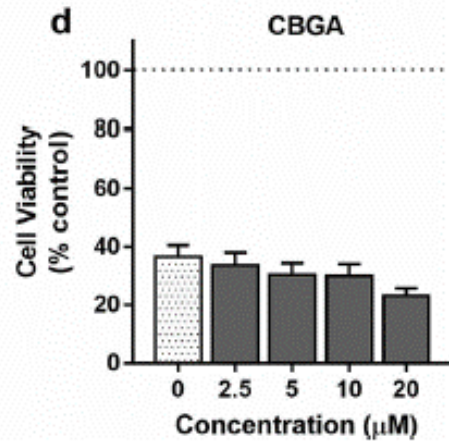
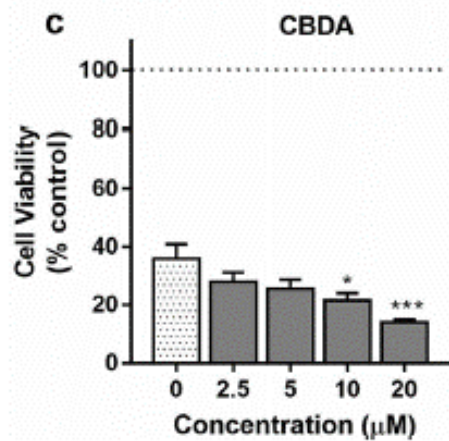
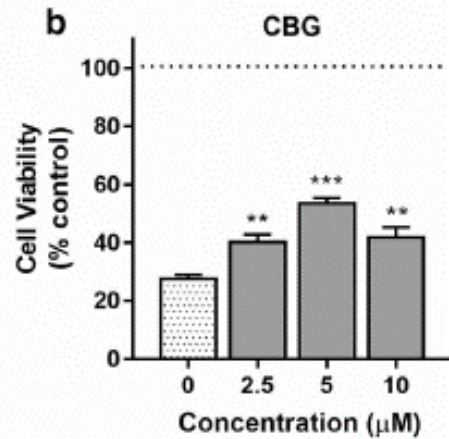
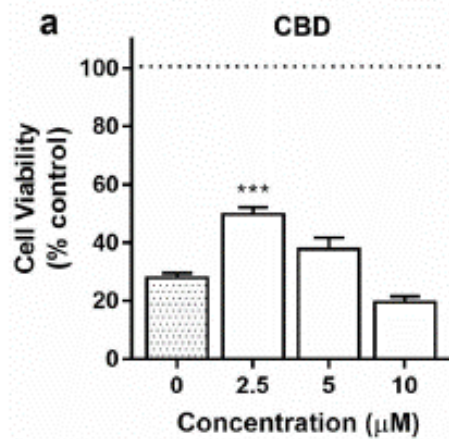


Neuroprotection assay against H₂O₂

Tubulina/Hoechst

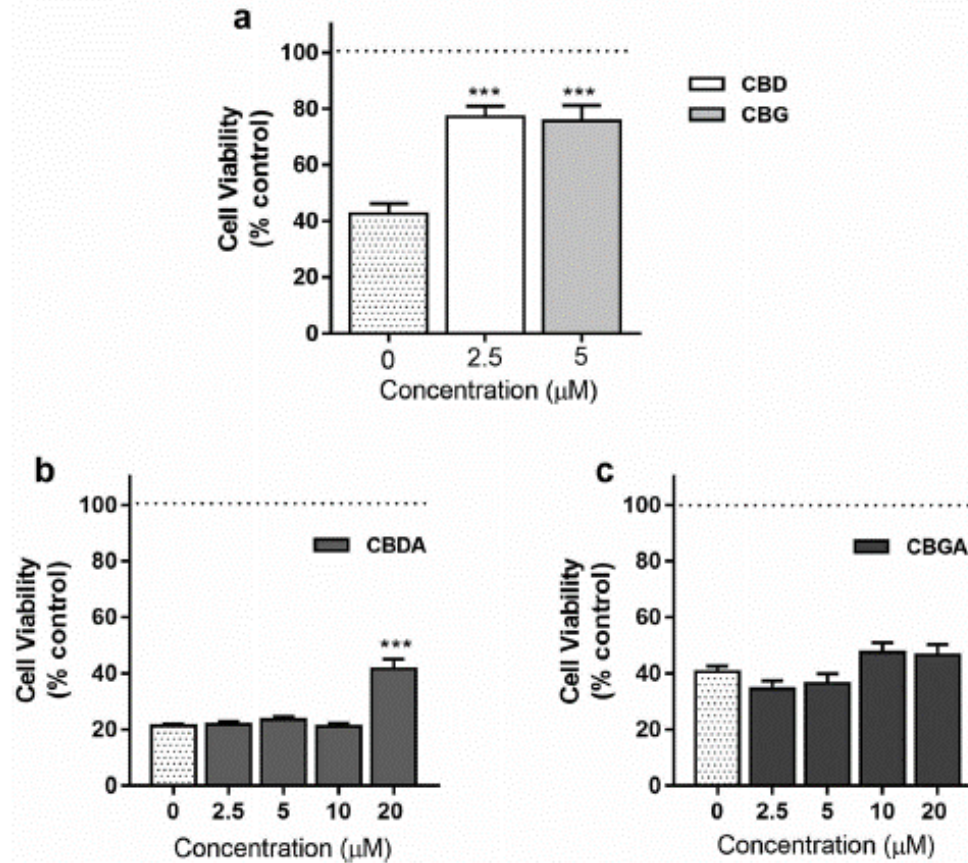


Cannabinoids neuroprotection - H₂O₂ (1 h)



Neuroprotection assay against rotenone

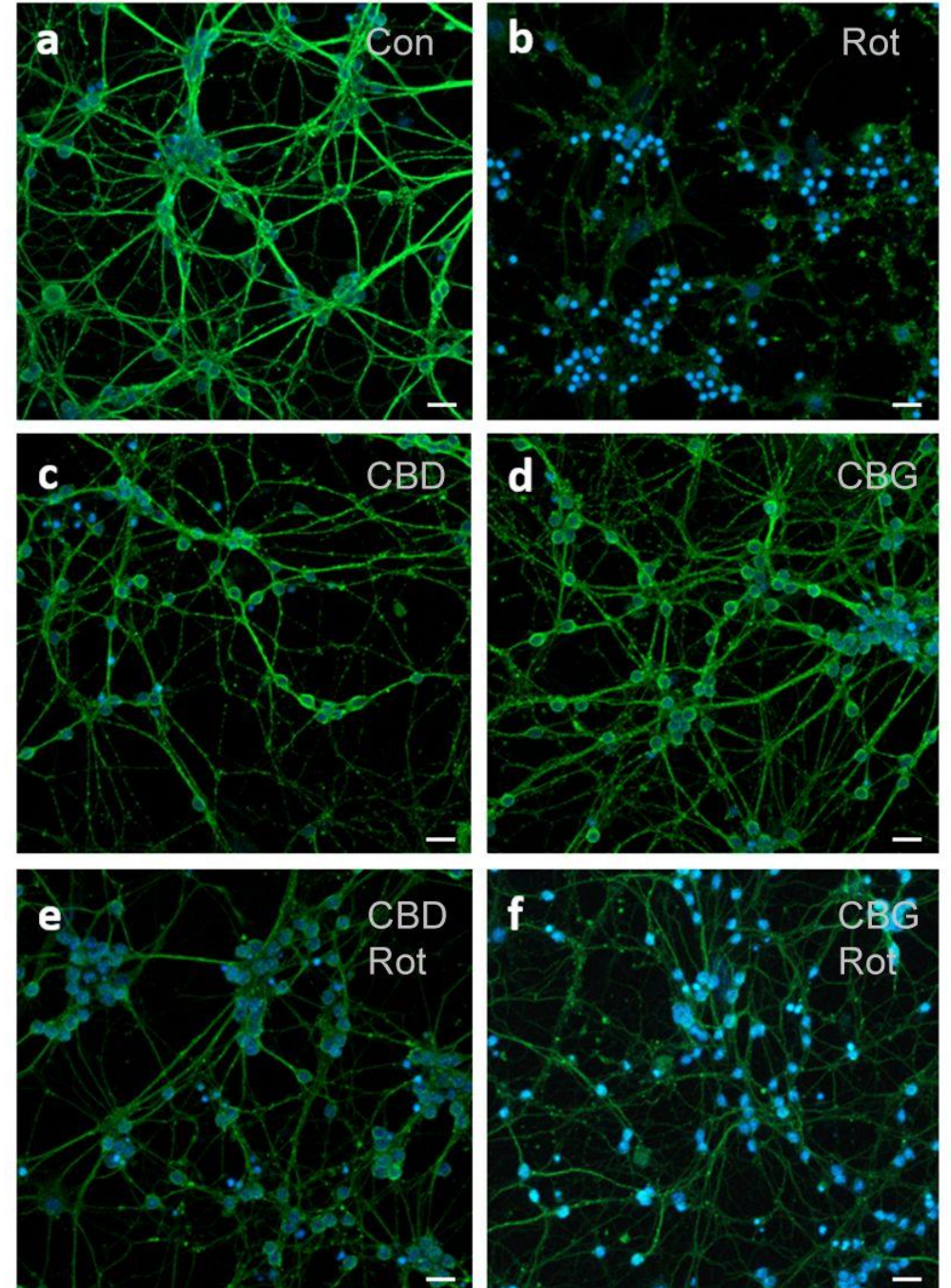
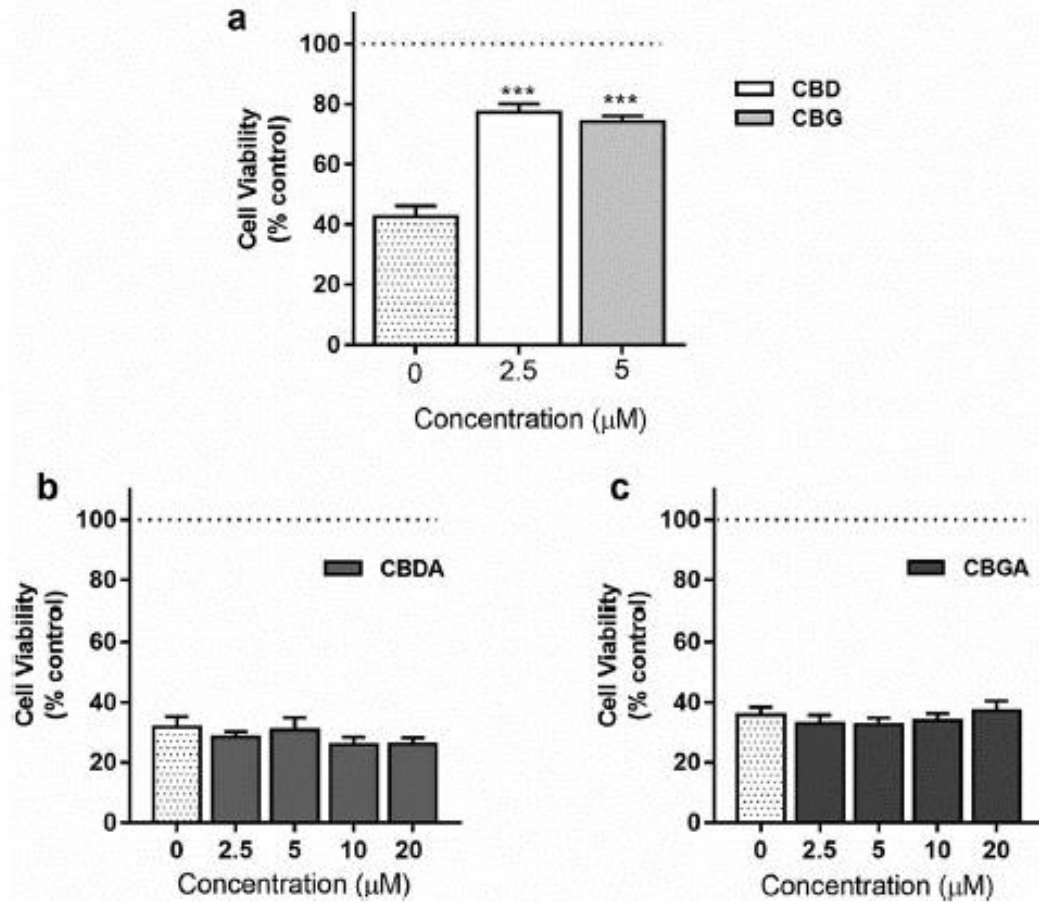
Cannabinoids neuroprotection - rotenone (24 h)



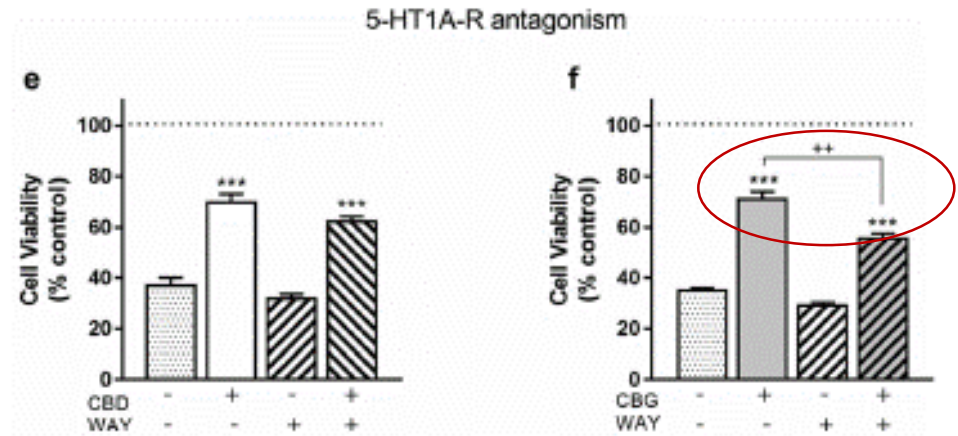
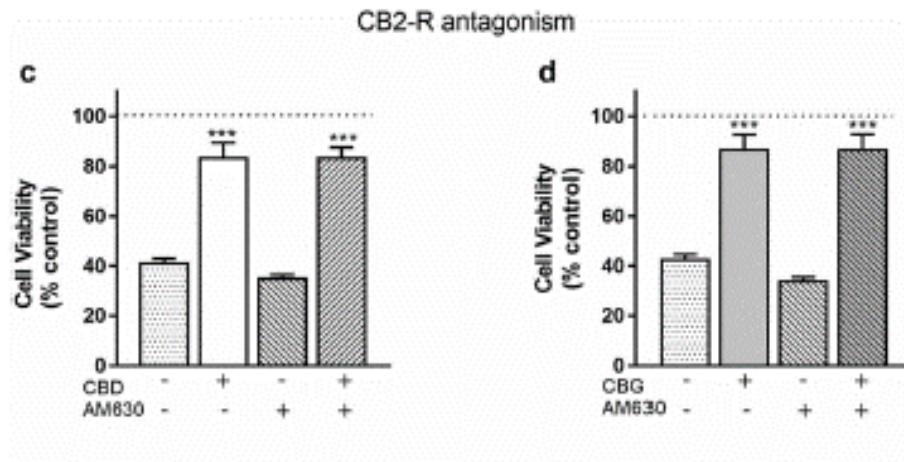
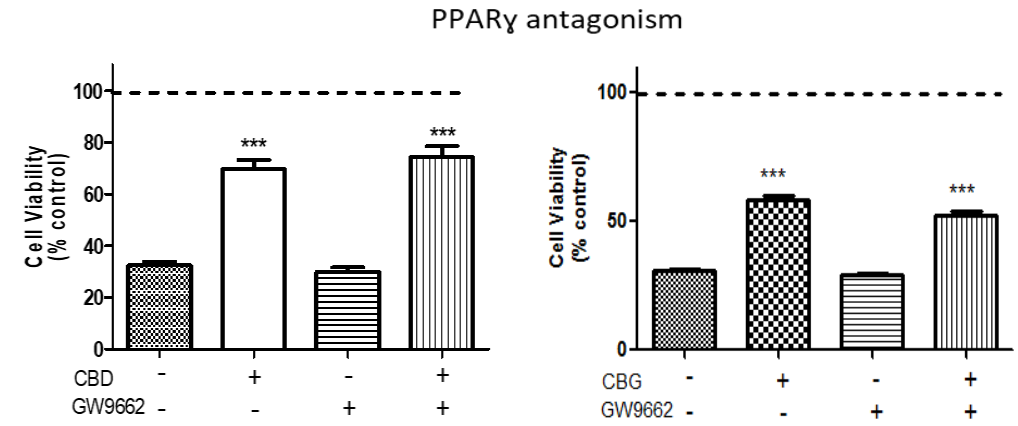
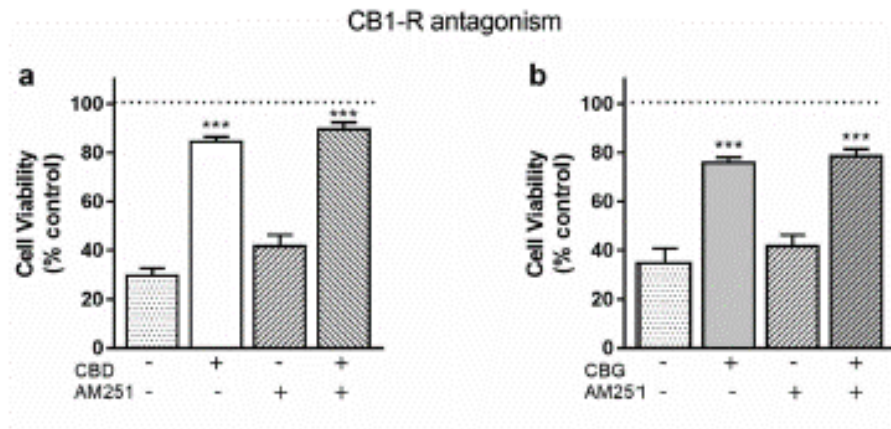
Neuroprotection assay against rotenone

Tubulina/Hoechst

Cannabinoids neuroprotection - rotenone (1 h)



Mechanism of action in rotenone assay





Neuroprotective agents

Isolated phytocannabinoids

Combined phytocannabinoids

Cannabis-based extracts:

Pharmaceutical products-with high content of CBD

Whole-plant cannabis extracts





Neuroprotective activity of combined phytocannabinoids

Given that the neuroprotective effect of CBD and CBG is independent CB1-R and CB2-R, their combination with THC could enhance their effects

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Proyecto de investigación Fundamental Fondo Clemente

Estable - 2020



DATOS GENERALES

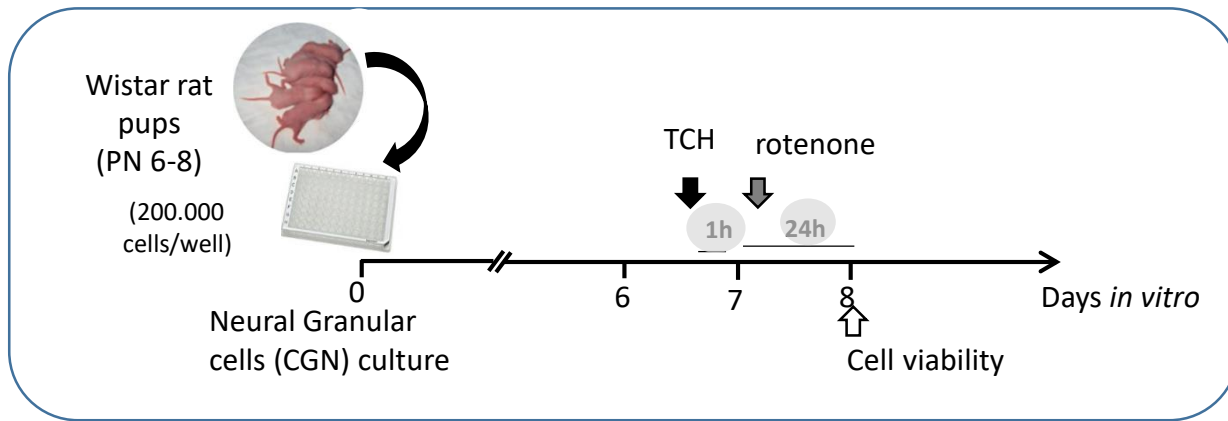
DATOS GENERALES

Título en español

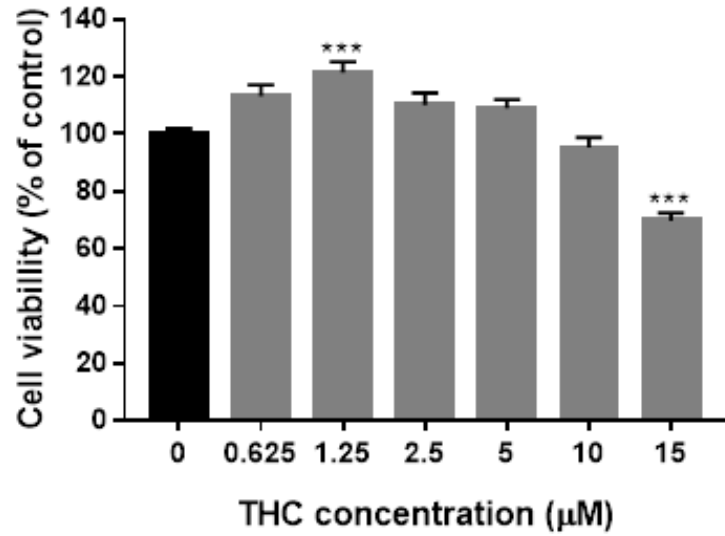
Uso medicinal de Cannabinoides: búsqueda de una combinación THC:CBD o THC:CBG que potencie su acción como agentes neuroprotectores y mejore su biodisponibilidad cerebral

Título en inglés

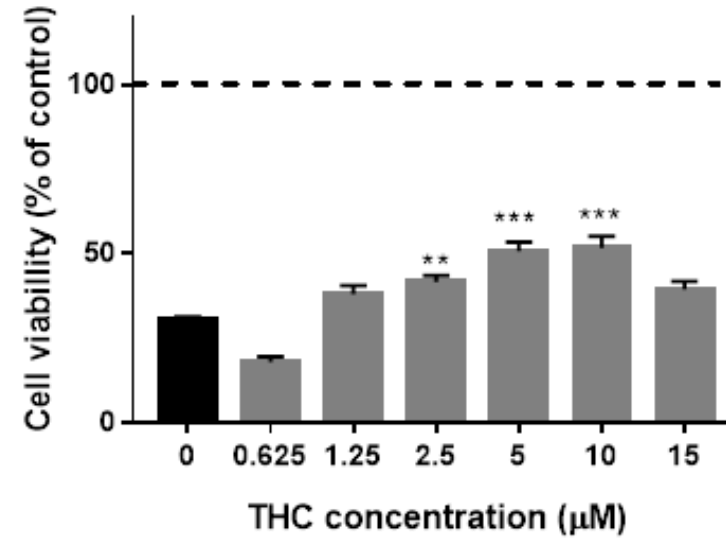
Cannabinoids medicinal use: search for a combination of THC:CBD and THC:CBG which potentiate their actions as neuroprotector agents and improve its brain bioavailability

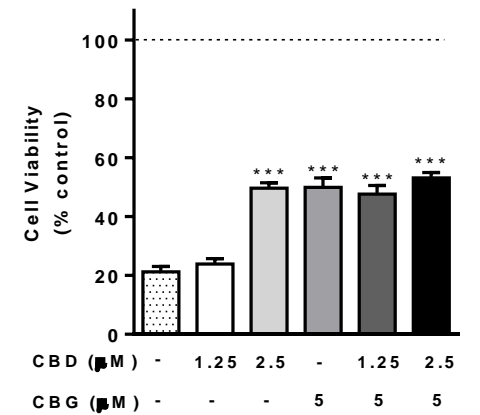
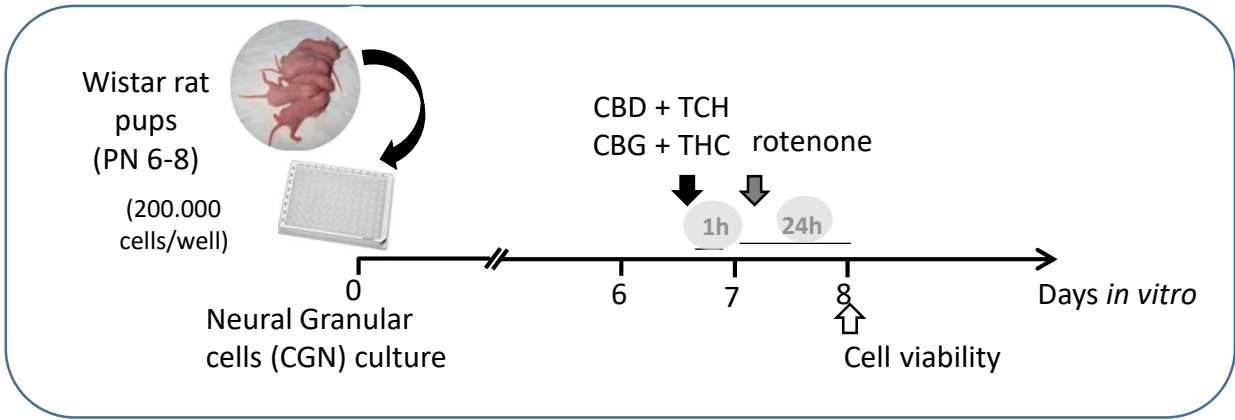


THC neurotoxicity

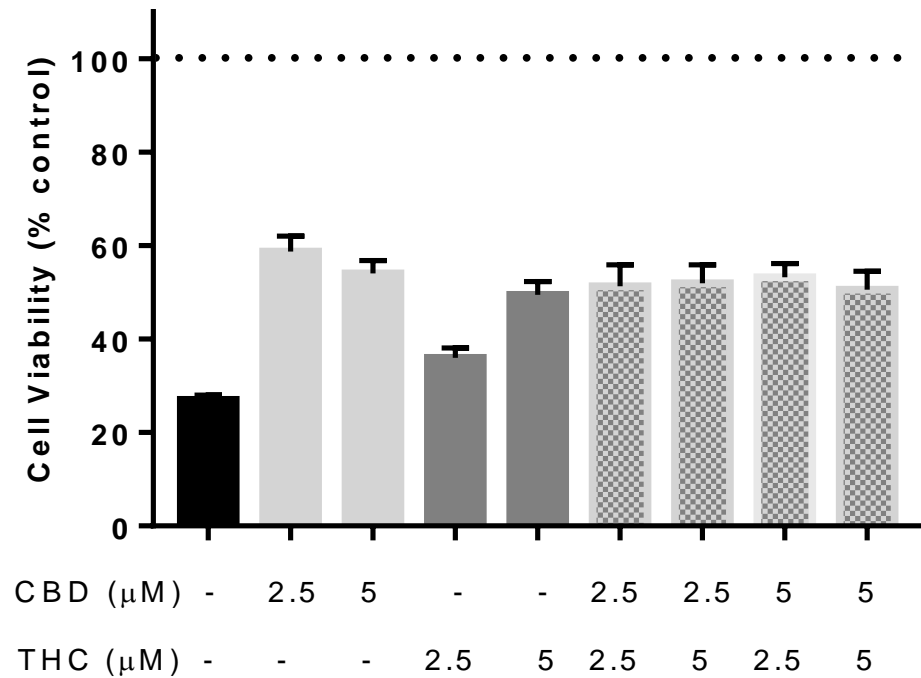


THC neuroprotection against rotenone

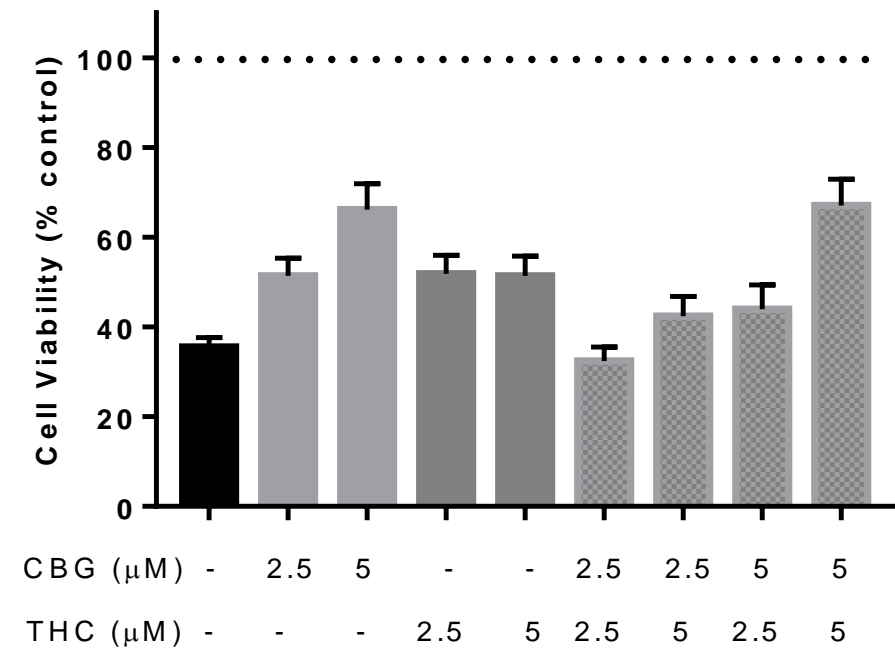




CBD + THC Neuroprotection



CBG + THC Neuroprotection





Neuroprotective agents?

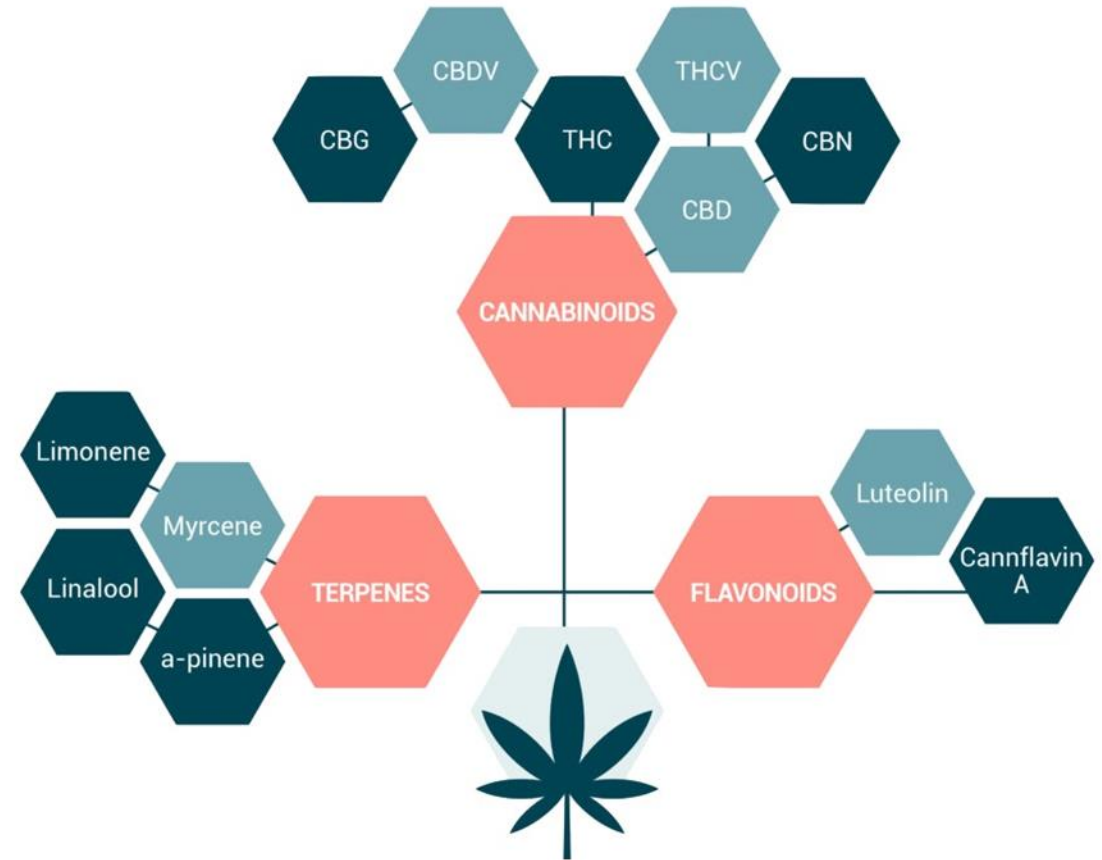
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Combined phytocannabinoids

Cannabis-based extracts:

Pharmaceutical products-with high content of CBD

Whole-plant cannabis extracts

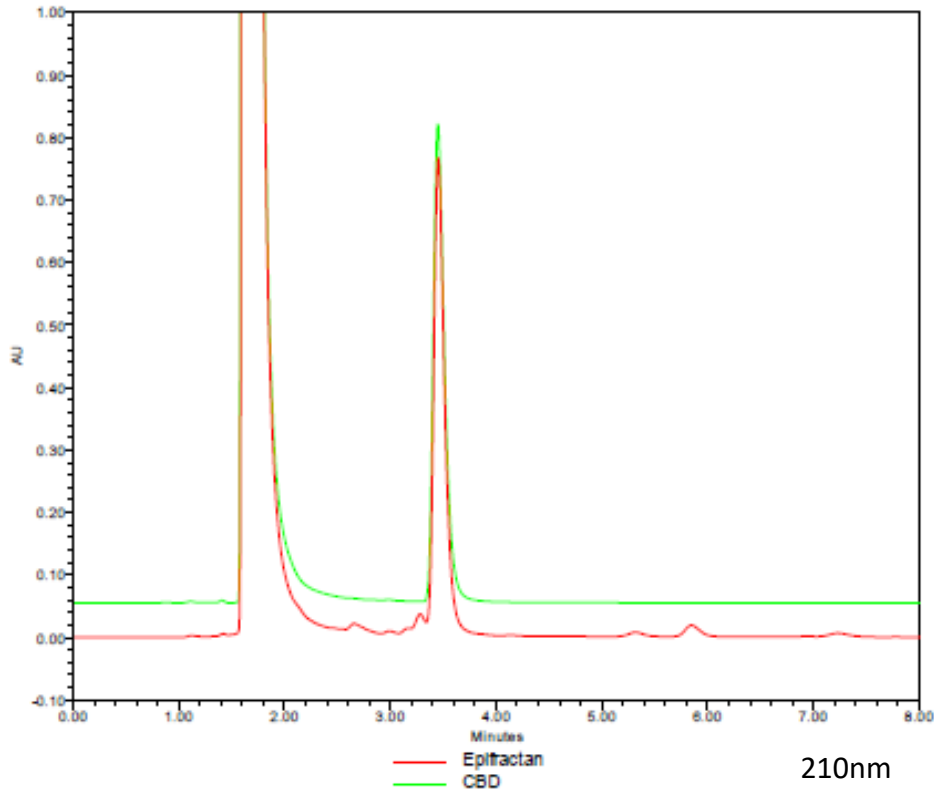


Entourage effect?

Neuroprotective activity of pharmaceutical cannabis-based extracts-with high content of CBD

First Uruguayan medical cannabis product registered (2017, 2%, 2018, 5%)

Registered only for the treatment of refractory epilepsy in children and adolescents



EPIFRACTÁN



5%
EXTRACTO DE
CANNABIS SATIVA L

SOLUCIÓN ORAL VÍA ORAL
DE ABSORCIÓN RÁPIDA
Uso en niños y adolescentes

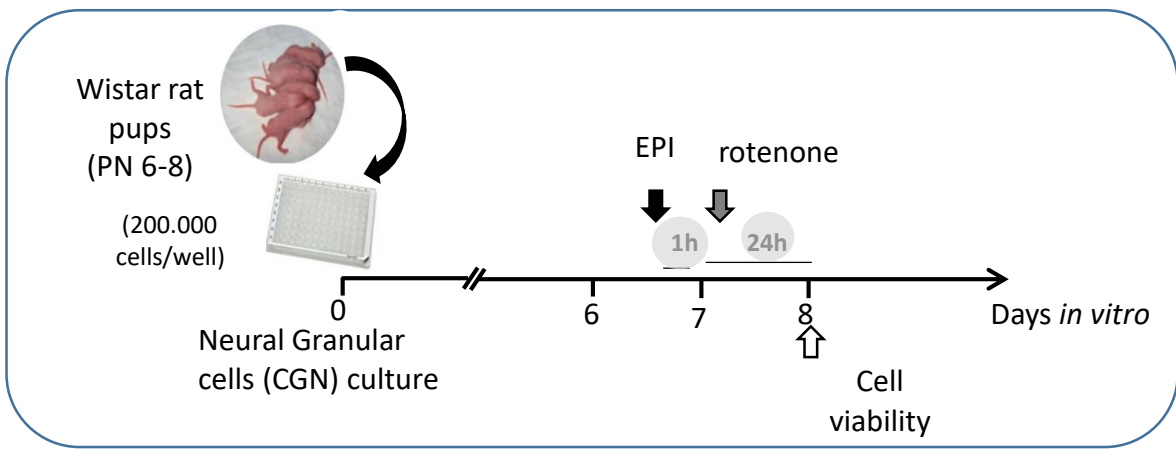


Uso
R A M M
P H A R M A

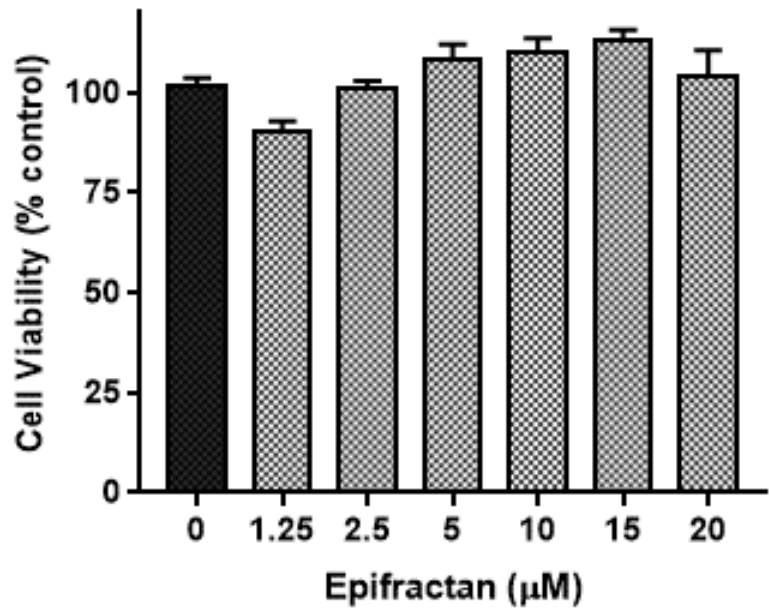
Fórmula:
Cada 100mL contiene:
Extracto de Cannabis Sativa L 100 g (*)
Excipientes c.s.
(*) Equivalente a 5 gramos de Cannabidiol.

Contiene menos de 0,2% de (THC + THCA)

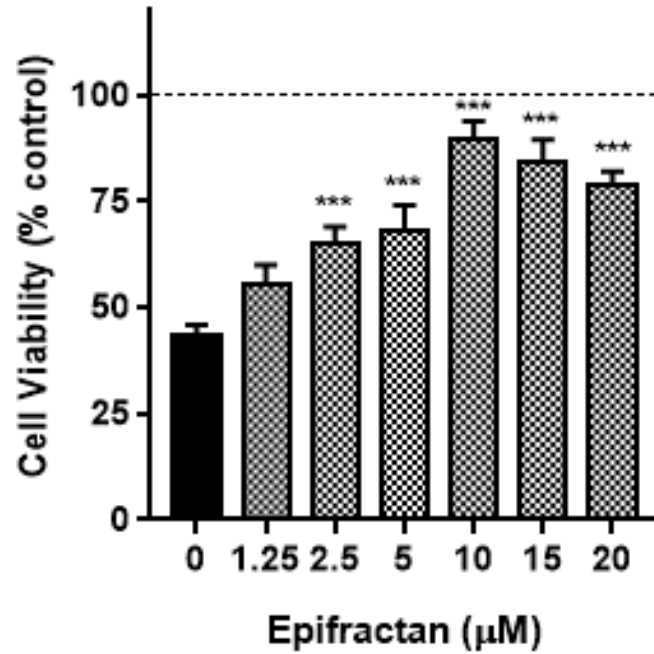
Acción terapéutica: Anticonvulsivante.
Indicaciones: Epilepsia Refractaria en niños y adolescentes.



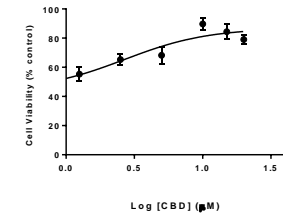
EPI Neurotoxicity



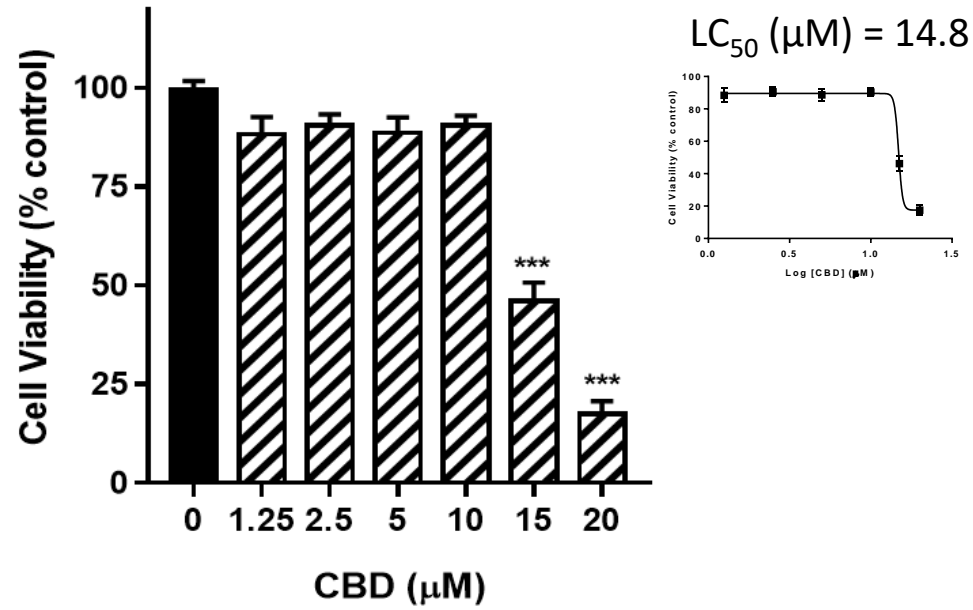
EPI Neuroprotection



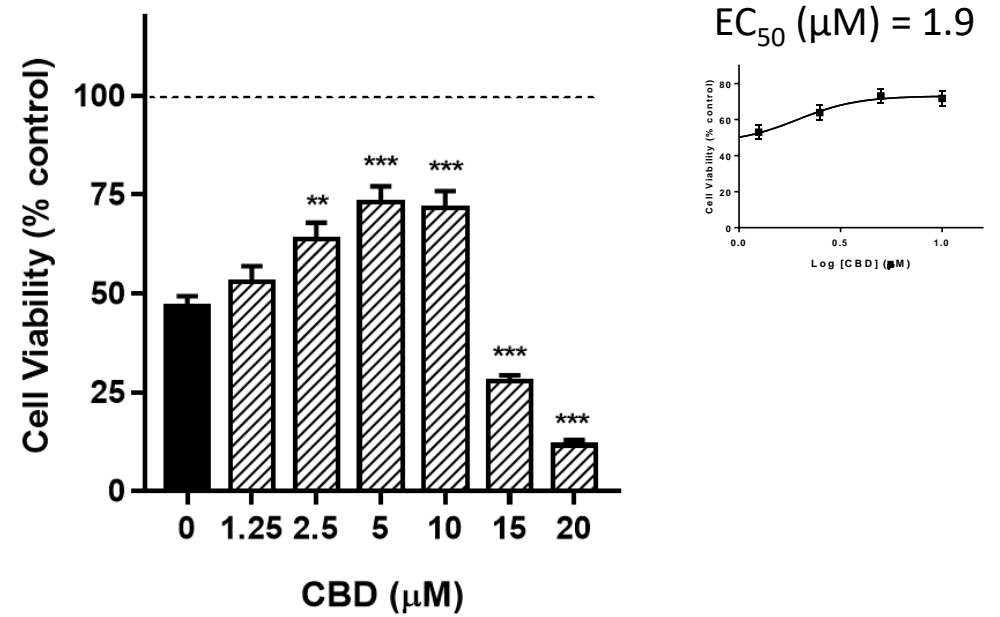
EC₅₀ (µM) = 2.6



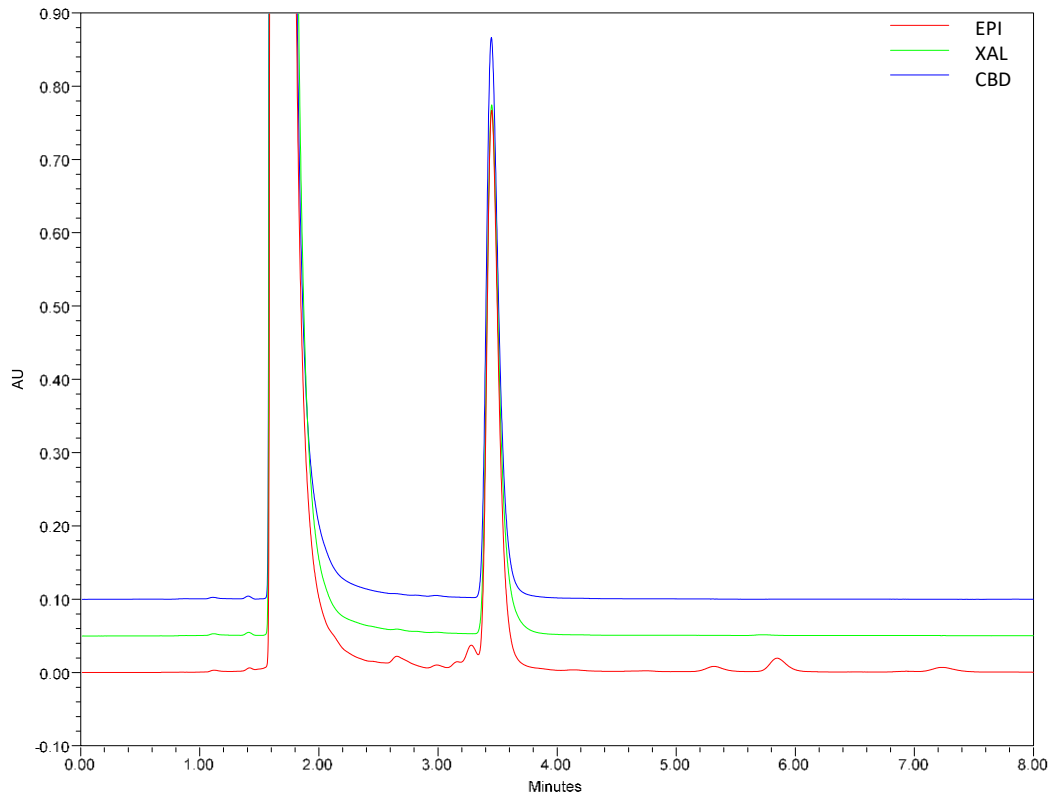
CBD Neurotoxicity



CBD Neuroprotection



XALEX 10% Pharmaceutical CBD purified of EPI



XALEX 10 10% CANNABIDIOL

Xalex 10™ es una solución al 10% de CBD puro de origen natural.
Contiene 100 mg de Cannabidiol por ml.
 No contiene THC.
 1 ml equivale a 34 gotas
 1 gota contiene 2,94 mg de CBD.

INDICADO PARA:

- Epilepsia refractaria en niños y adolescentes.
- Epilepsia refractaria en adultos.
- Dolor crónico.
- Espasticidad por esclerosis múltiple.
- Trastornos neurodegenerativos, neuroprotector.
- Trastornos asociados al espectro autista.
- Náuseas y vómitos inducidos por quimioterapia.
- Trastornos del sueño, ansiolítico.
- Tratamiento de abuso de sustancias, psicosis y depresión.

TRATAMIENTO MÉDICO:

Venta bajo receta médica.
 La prescripción médica de CBD la realiza el médico general y el especialista en receta común.
 El CBD no es psicotrópico.
 La indicación es individual y la dosis varía según la persona, la afección y la evolución del tratamiento.
 La dosis individualizada se identifica al lograrse el efecto buscado.
 Se recomienda seguimiento médico.

DOSIFICACIÓN:

La titulación de la dosis debe ser individualizada y supervisada por el médico tratante.

Recomendación:
 Dosis de inicio: 0,1 - 0,5 mg/kg/día
 Dosis máxima: 20mg/kg/día

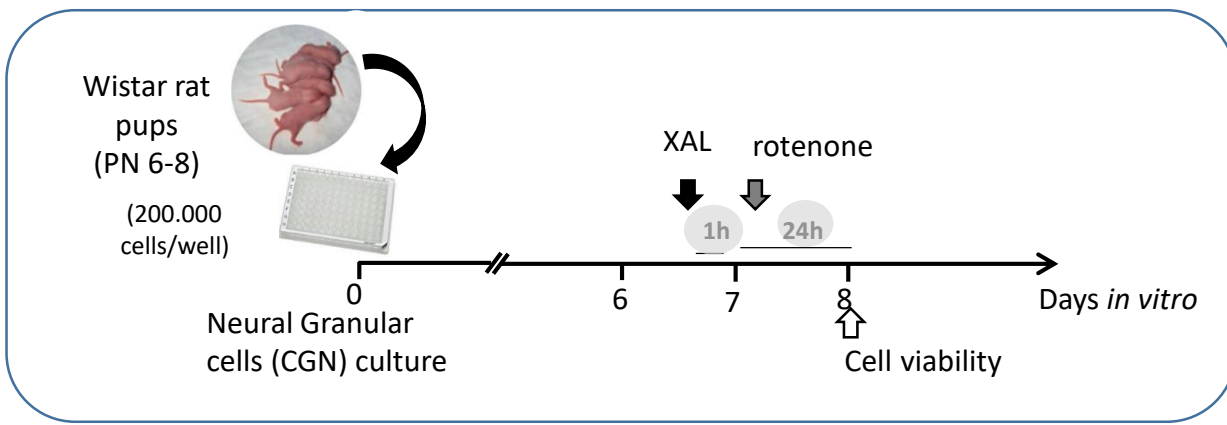
Ver ejemplo de esquema de titulación:

Xalex10	Mañana (gotas-mg)	Noche (gotas-mg)	mg/kg/día Peso: 10kg	mg/kg/día Peso: 30kg	mg/kg/día Peso: 50kg	mg/kg/día Peso: 70kg	mg/kg/día Peso: 100kg
Día 1		3 gotas - 8,82mg	0,88	2,92	0,18	0,13	0,09
Día 4		5 gotas - 14,7mg	1,47	4,41	0,29	0,21	0,15
Día 8	3 gotas - 8,82mg	5 gotas - 14,7mg	2,35	7,05	0,41	0,29	0,21
Día 12	5 gotas - 14,7mg	8 gotas - 23,5mg	3,74	11,22	0,67	0,47	0,33
Día 20	8 gotas - 23,5mg	10 gotas - 29,4mg	5,41	16,23	0,98	0,69	0,49
Día 30	10 gotas - 29,4mg	15 gotas - 44,1mg	7,35	22,05	1,36	0,96	0,69
Día 45	15 gotas - 44,1mg	20 gotas - 58,8mg	9,80	29,40	1,84	1,30	0,94

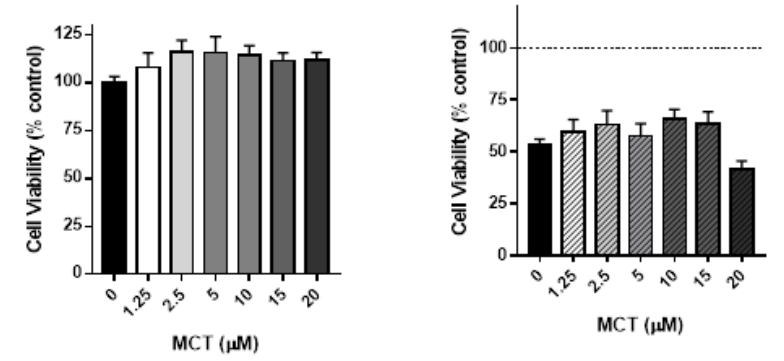
Gotas	CBD (mg)	Peso: 10Kg (mg/kg/día)	Peso: 30Kg (mg/kg/día)	Peso: 50Kg (mg/kg/día)	Peso: 70Kg (mg/kg/día)	Peso: 100Kg (mg/kg/día)
3	8,82	0,88	2,92	0,18	0,13	0,09
5	14,70	1,47	4,41	0,29	0,21	0,15
7	20,58	2,06	6,27	0,41	0,29	0,21
10	29,40	2,94	8,82	0,59	0,43	0,31
15	44,10	4,41	13,23	0,88	0,63	0,44
20	58,80	5,88	17,64	1,16	0,84	0,59
30	88,20	8,82	26,46	1,74	1,26	0,88
40	117,60	11,76	35,28	2,32	1,68	1,18
50	147,00	14,70	44,10	2,90	2,10	1,47
60	176,40	17,64	52,92	3,48	2,52	1,76
70	205,80	20,58	61,74	4,06	2,94	2,06
80	235,20	23,52	70,56	4,64	3,36	2,35
90	264,60	26,46	79,38	5,22	3,78	2,65
100	294,00	29,40	88,20	5,80	4,20	2,94

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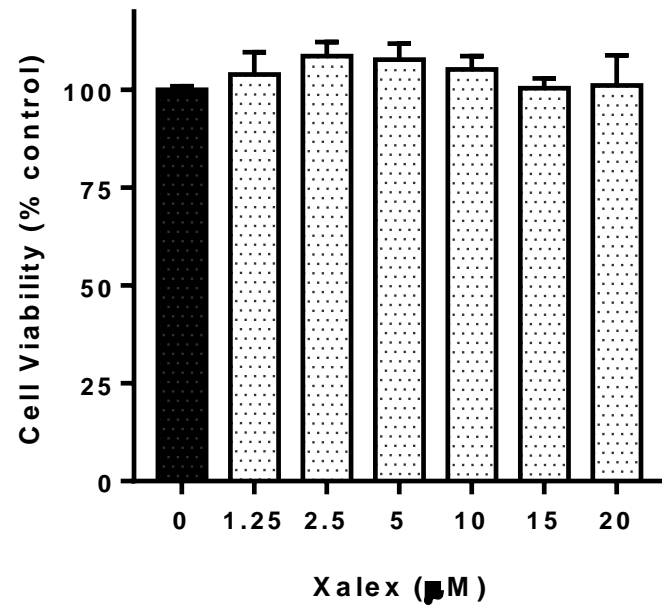
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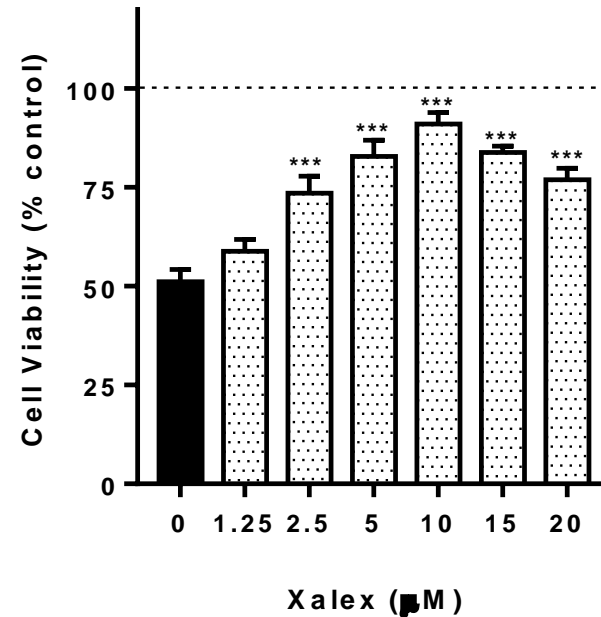
MCT (medium-chain triglyceride)



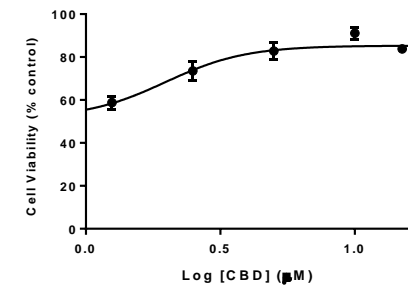
XAL Neurotoxicity



XAL Neuroprotection



EC₅₀ (μM) = 1.9





Neuroprotective agents?

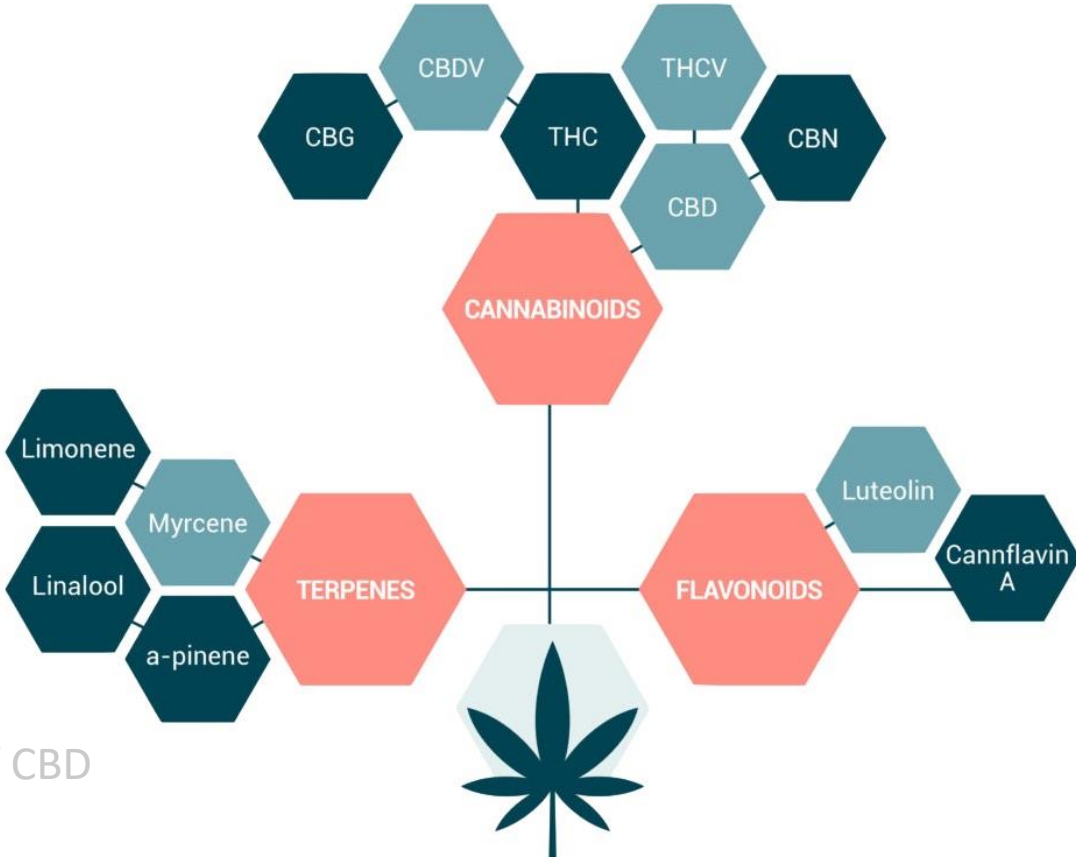
Isolated phytocannabinoids

Combined phytocannabinoids

Cannabis-based extracts:

Pharmaceutical products-with high content of CBD

Whole-plant cannabis extracts



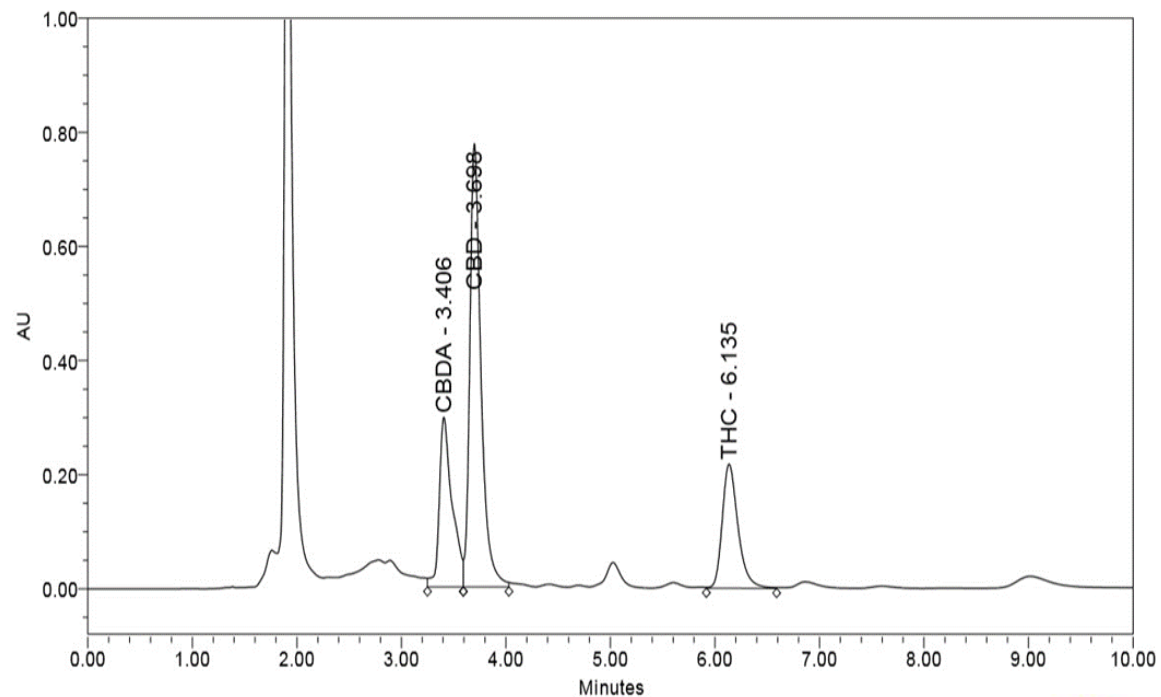
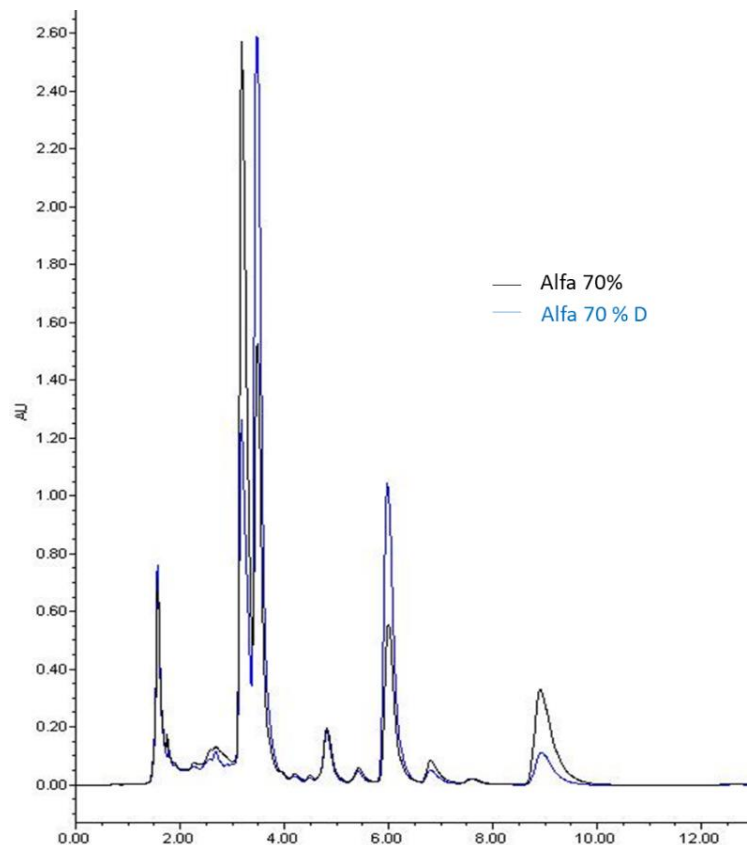
Entourage effect?

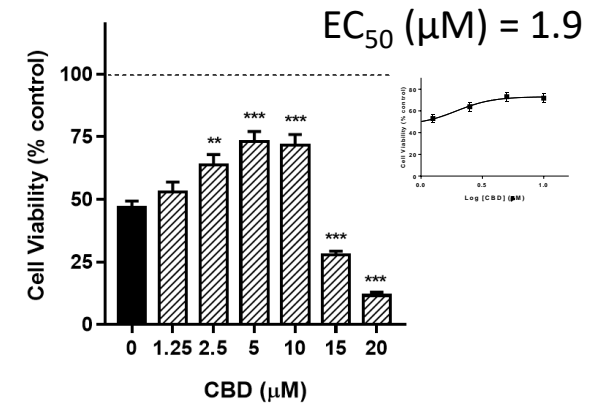
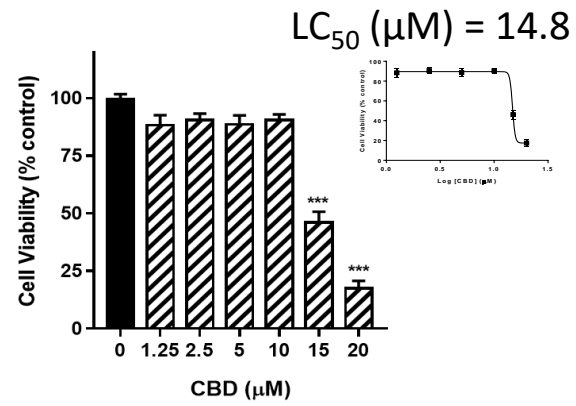
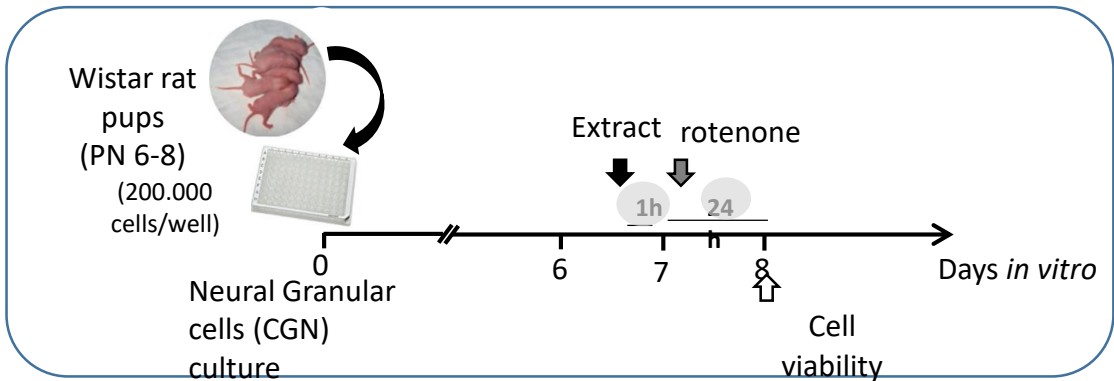
Neuroprotective effect of Cannabis extracts (alfa y beta varieties)



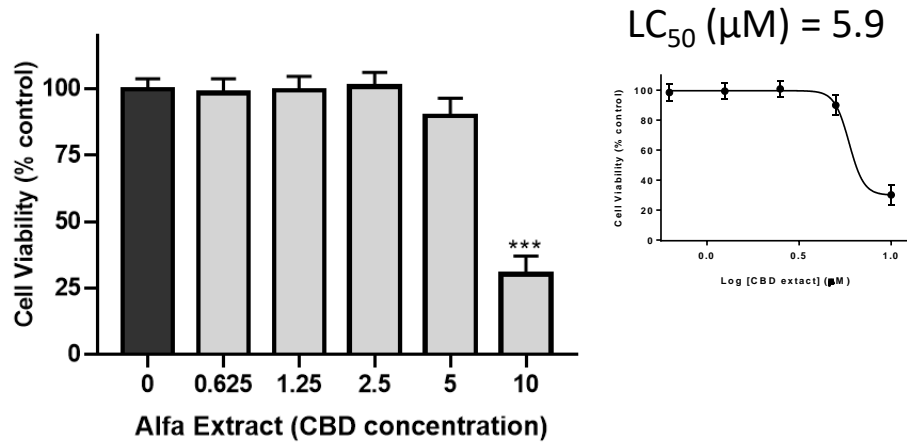
F. Vignolo

- EtOH-H₂O extracts
- Decarboxylation (120 min, 90 °C)
- Analysis of Cannabinoid Content (CBD, CBDA ,THC) - HPLC-DAD (210nm)

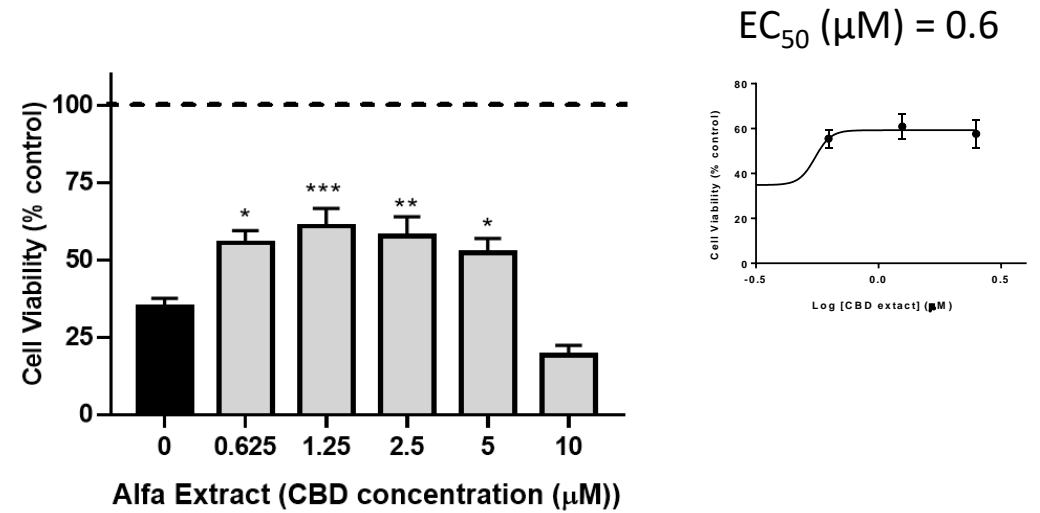




Extract neurotoxicity



Extract neuroprotection



Conclusions

- ✓ Our results support the notion that cannabis extracts possess differential pharmacological properties compared to isolated cannabinoid molecules
- ✓ There is a synergistic effect between the different Cannabis compounds for both neuroprotection and neurotoxicity
- ✓ In vivo studies are necessary in this topic



NeuroCann

THANK YOU

For your attention!!!

Acknowledgments



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de Educación
y Cultura



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