UNIVERSITY **OF MIAMI**

Introduction

Phantom Limb Pain (PLP) often results from medically required limb amputation and becomes difficult to manage due to underlying inflammation and other neuropathologies, affecting up to 85% of amputees. Medical marijuana is often used for pain relief and may be beneficial for chronic pain syndromes like PLP due to the wealth of cannabinoid (CB) compounds acting via distinct or synergistic mechanisms. The goal of this study was to evaluate the analgesic potential of delta-9-tetrahydrocannabinol (THC), Cannabidiol (CBD), βcaryophyllene (BCP) and their combination in preventing or reversing PLP-like behavior.

Methods

Animals: Male rats Sprague Dawley, 140g. **Surgeries:** All surgeries under anesthesia, rats allowed to fully recover before returned to the home cages. Formalin injections: 50 ul of 5% formalin was injected into lateral side of the plantar hind paw. Chronic constriction injury (CCI): Sciatic nerve was exposed and 4 chromic gut loose ligatures placed around the nerve (Bennett, 1996). Axotomy: Sciatic nerve was exposed, tightly ligated with silk and transected below the ligature. **Drug treatment:** Animals were assigned to 1 of 4 treatment groups and received treatments twice daily. CBD/BCP (2.0 mg/kg:16 mg/kg), CBD/BCP/THC (2.0 mg/kg:16 mg/kg:0.04 mg/kg), THC (0.04 mg/kg). **Behavior:** Animals were observed and scored daily. Scoring scale: 1 point (p) for nail biting (Level I, max 5p), 3p for injury of the distal digit (Level II, starting at 5p, 20p max), 5p for injury of proximal digit (Level III, starting at 20p, cut off 25p). Animals are sacrificed when proximal injury appears, with the day of termination recorded, or by 72 days post-axotomy. Final scores were retained until the end of the experiment. Graph Pad and Sigma Stat software used for behavioral evaluations. **Tissue processing:** Perfusion with 4% paraformaldehyde, tissue cryoprotected with 30% sucrose and cryosectioned. **ELISA:** Animals euthanized by CO2, fresh spinal tissue removed, snap frozen in dry ice and stored at -80C. Protein concentration in the homogenized tissue determined by BCA (Thermo Scientific) and processed for ELISA according to manufacturer's protocol (Abcam, RayBiotech).



Evaluation of Cannabis constituents in a model of phantom limb pain in rats

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controls that received only vehicle. Further, the group that received THC alone had significantly higher GAD concentration than the combined CBD/BCP/THC group. This upregulation suggests restoration of GABA production and spinal inhibition following cannabinoid treatments in injured animals, as decreased dorsal horn GABA function has been associated with neuropathic pain. *p<0.05, **p<0.01 vs PLP control #p<0.05 between groups.





Levels of anti-inflammatory cytokines, IL-4 and IL-10, in lumbar spinal tissue were significantly upregulated in animals treated with CBD/BCP, CBD/BCP/THC or THC alone compared to vehicle controls. BDNF and TNF α in lumbar spinal tissue were significantly reduced in animals treated with CBD/BCP, CBD/BCP/THC or THC alone compared to vehicle controls. IL1B regulation was not significantly affected by either drug treatment. Between treatment groups, animals that received either CBD/BCP or THC alone had significantly higher levels of TNF α compared to animals that received the full cohort of drugs. *, ** p<0.05, 0.01 compared with control; #, ## p<0.05, 0.01 between CBD/BCP/THC and CBD/BCP or THC alone treatment.

- Administration of all the tested cannabis combinations showed attenuation in the severity and onset of PLP-like behaviors compared to the vehicle controls
- Comparison between experimental groups showed that animals treated with either
- THC alone or CBD:BCP combination displayed lower autotomy scores compared to animals receiving all three together (THC:CBD:BCP).
- All cannabinoid treatments produced increased anti-inflammatory cytokines IL-4 and IL-10 as well as decreased levels of BDNF and TNF α .
- All treatment groups showed significantly higher levels of GAD65/67 compared untreated injured PLP controls.