A novel cannabidiol:tetramethylpyrazine (CBD-TMP, ART12.11) co-crystal improves the efficacy and bioavailability of cannabidiol to induce anxiolytic and anti-depressant effects

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Introduction

Cannabidiol (CBD):

- CBD is a phytocannabinoid that develops from its precursor cannabidiolic acid, which is formed naturally in the *Cannabis sativa* plant.
- Many previous studies have demonstrated that CBD can reduce anxiety levels and induce anti-depressant effects in both acute and chronic dosing regimens [1,2,3].
  - Other notable functions include anti-inflammatory, anti-oxidant, anti-epileptic, and analgesic effects.
- Unlike ∆9-trans-tetrahydrocannabinol (THC) – the main phytocannabinoid present in the *Cannabis sativa* plant, CBD does not directly bind endocannabinoid receptors such as CB1r and CB2r [4,5]. This results in CBD having little-to-no psychotropic effects and minimal reinforcing properties.
- Although CBD offers much promise for therapy, it has drawbacks which include low bioavailability, inconsistencies in dosing, and solid-state polymorphisms.

Tetramethylpyrazine (TMP):

- TMP is an active component in the Chinese herb *Ligusticum Chuanxiong Hort*, which is often used in various forms of Asian traditional medicine.
- Previous reports have shown TMP is able to reduce anxiety in stressed rodent models, has potential anti-depressant activity, and displays protective effects for learning and memory [6,7,8].
  - TMP displays neuroprotective, anti-oxidant, anti-apoptosis, anti-inflammatory, and anti-ischemic properties [9,10].
- TMP can effectively cross the Blood-Brain-Barrier [11].

CBD-TMP co-crystal (ART 12.11):

- A CBD-TMP co-crystal (ART 12.11) was recently developed to combat problems associated with pharmacotherapeutic CBD dosing and administration.
- Co-crystallization offers many advantages, including better bioavailability, improved stability, greater dose-to-dose consistency, and reduced solid-state polymorphisms.
- **The goal of this research project is to investigate and characterize the potential therapeutic effects of ART12.11.**

Methods

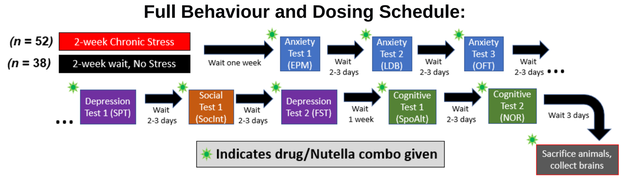
Behaviour studies: Adult male Sprague Dawley rats (N = 90) were divided into non-stressed (*n* = 38) and stressed (*n* = 52) branches, where stressed animals underwent a two-week chronic unpredictable stress protocol as described below. Following conclusion of the stress protocol, all animals went through a series of behaviour tests examining anxiety, depression, and cognition.
Drugs were mixed into Nutella (6 g/kg) and administered orally 2 hours before each behavioural test.

Two-Week Chronic Unpredictable Stress Protocol:

Day	1	2	3	4	5	6	7
Stressor 1	Cage tilt (2h)	Wet bedding (4h)	Light deprivation (3h)	Restraint stress (1.5h)	Foot shocks (x5, 0.7 mA)	Wet bedding (4h)	Cage tilt (2h)
Stressor 2	Restraint stress (1.5h)	Overnight food deprivation	Cage tilt (2h)	Foot shocks (x5, 0.6 mA)	Overnight food deprivation	--	Light deprivation (3h)

8	9	10	11	12	13	14
Foot shocks (x5, 0.7 mA)	Restraint stress (2h)	Wet bedding (4h)	Wet bedding (4h)	Cage tilt (2h)	Restraint stress (2.5h)	Wet bedding (4h)
Light deprivation (3h)	Overnight food deprivation	Cage tilt (2h)	Light deprivation (3h)	Foot shocks (x5, 0.8 mA)	Overnight food deprivation	Light deprivation (3h)

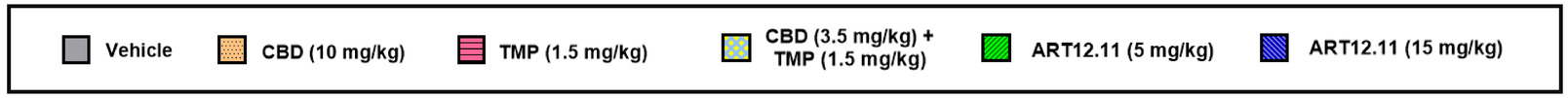
Full Behaviour and Dosing Schedule:



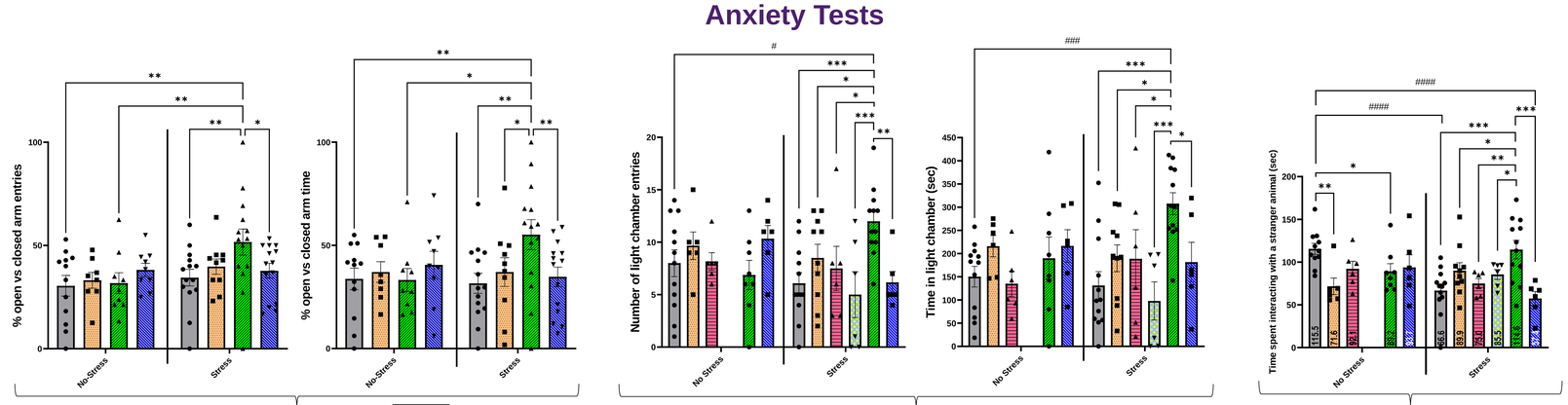
Pharmacokinetic studies: Adult male beagle dogs were (N = 6) were fed either CBD (10 mg/kg) or ART12.11 in capsules in fed or fasted states throughout a 7-day period. Blood plasma levels of CBD and its metabolite (CBD-7COOH) were taken for 12 hours after dosing to test the bioavailability of ART12.11.

Data Analysis: Data is presented as mean ± SEM. Data were first analyzed with Bartlett's test to confirm equal standard deviation, then analyzed using one-way or two-way ANOVA where appropriate. Post-hoc analyses were calculated using Fischer's LSD (represented with *). Two-tailed t-tests were done to compare Vehicle (No-Stress) group with Stress groups when two-way ANOVA were not possible (represented with #). The significance level was set to *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. Outliers were identified and removed using Grubbs Outlier test (*p<0.05).

Behavioural Results



Anxiety Tests

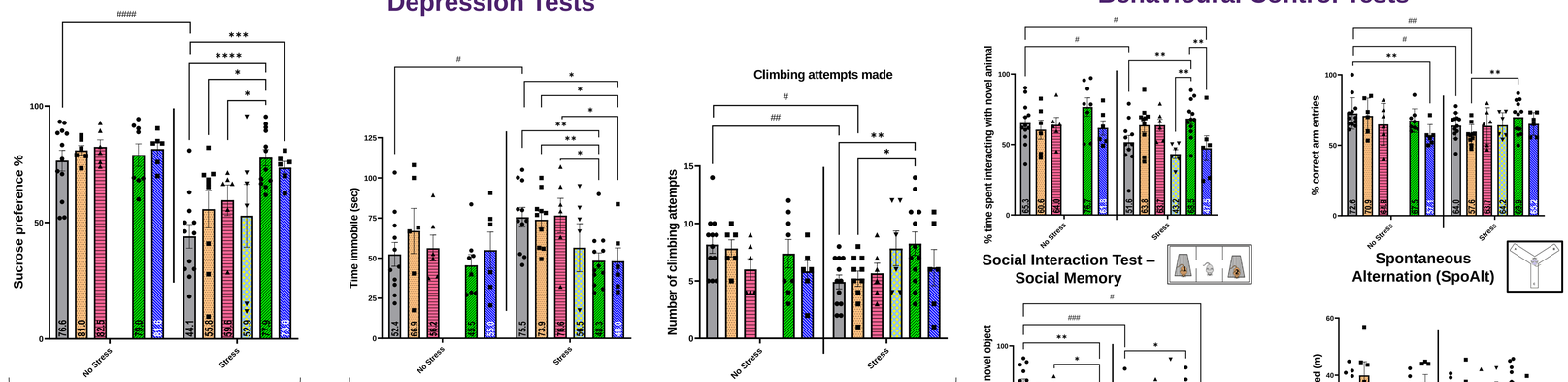


Elevated-Plus Maze (EPM)

Light-Dark Box (LDB)

Social Interaction Test – Social Motivation

Depression Tests



Sucrose Preference Test (SPT)

Forced-Swim Test (FST)

Behavioural Control Tests

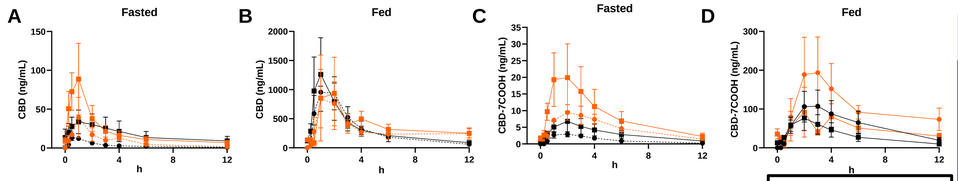
Social Interaction Test – Social Memory

Spontaneous Alternation (SpoAlt)

Novel Object Recognition (NOR)

Open-Field Test

Pharmacokinetic Results



Pharmacokinetic profiles of ART12.11 (10 mg/kg) and CBD (10 mg/kg) in fed and fasted beagle dogs taken after the first (day 1) and seventh (day 7) dose of the drug. CBD levels are presented in A and B, and CBD metabolite (CBD-7COOH) levels in C and D.

References

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Funding:
Artelo Biosciences
Mitacs
NSERC CRSNG

Conclusions

- ART12.11 (5 mg/kg) reduced anxiety in stressed animals.
  - In the Social Interaction test, ART12.11 (5 mg/kg) reversed the stress-mediated increases in social anxiety.
- ART12.11 (5 mg/kg) had anti-depressant effects and reversed the stress-mediated effects on depression, including reduced anhedonia and reduced learned helplessness behaviours.
- ART12.11 (5 mg/kg) did not negatively impact social memory, spatial memory, short-term memory, or gross motor behaviour.
- The anxiolytic and anti-depressant effects noted with ART12.11 were not observed upon administration of CBD alone, TMP alone, or a mixture dose of CBD and TMP together.
- ART12.11 improves the oral bioavailability of CBD in the fasted state.
- These data suggest ART12.11 is a promising and superior alternative to CBD in the pharmacotherapeutic treatment of anxiety and depressive symptoms and deserves further investigation of pharmacotherapeutic potential.