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ORIGINAL INVESTIGATION

Early oral administration of THC:CBD formulations prevent pain-related behaviors without exacerbating paclitaxel-induced changes in weight, locomotion, and anxiety in a rat model of chemotherapy-induced neuropathy

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Abstract

Rationale Paclitaxel-induced neuropathy stands out as the primary, dose-limiting side effect of this extensively used chemotherapy agent. Prolonged hypersensitivity and pain represent the most severe clinical manifestations. Effective preventive and therapeutic strategies are currently lacking.

Objectives Our study aimed to assess the impact of early oral administration of pharmaceutical-grade formulations containing the phytocannabinoids THC and CBD in a rat model of paclitaxel-induced neuropathy.

Methods The experimental design involved the co-administration of paclitaxel and cannabinoid formulations with different THC to CBD ratios (THC:CBD 1:1 and THC:CBD 1:20) to adult male rats. Mechanical and thermal sensitivity, locomotor activity, vertical exploratory behaviors, anxiety-related parameters, weight gain, food and water consumption, and liver functionality were assessed.

Results Daily administration of THC:CBD 1:1 successfully prevented paclitaxel-induced cold allodynia, while THC:CBD 1:20 effectively prevented both thermal and mechanical hypersensitivities. Additionally, THC:CBD 1:1 formulation restored rearing behavior, significantly reduced by paclitaxel. Conversely, neither cannabinoid formulation was able to counteract paclitaxel-induced hypo-locomotion, reduced vertical exploratory activity, increased anxiety-like behaviors, attenuated weight gain, or decreased food and water intakes. However, the formulations employed did not induce further alterations or toxicity in animals receiving paclitaxel, and no signs of liver damage were detected.

Conclusions Our results suggest a differential therapeutic effect of two THC:CBD formulations on pain-related behaviors and spontaneous activities, particularly in the context of peripheral neuropathy. These formulations represent a promising therapeutic strategy not only to managing pain but also for enhancing daily activities and improving the quality of life for cancer patients.

Keywords Paclitaxel-induced behavioral changes · Cannabinoid formulations · THC:CBD ratios · Cannabinoid therapeutic actions · Drug-induced neurotoxicity · Spontaneous behaviors in rodents · Pain management · Ethological behaviors · Anxiety-like behaviors · Locomotor activity

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Introduction

Paclitaxel, a chemotherapeutic agent widely used in the treatment of various cancers, is notably effective but often limited by its severe side effects, particularly peripheral neuropathy (Burgess et al. 2021; Cimbro et al. 2024; Kurt et al. 2023). This condition, characterized by symptoms such as numbness, tingling, and persistent pain, represents the most significant dose-limiting side effect in paclitaxel-based regimens (Cimbro et al. 2024; Nyrop et al. 2019; Soriano et al. 2024). Approximately 40–60% of patients receiving paclitaxel experience neuropathy, which can persist long after treatment completion, severely impacting quality of life and sometimes leading to discontinuation of a potentially life-saving therapy (Cimbro et al. 2024; Nyrop et al. 2019; Soriano et al. 2024). In addition, patients receiving paclitaxel may experience motor impairments, cognitive difficulties, and mood disorders such as depression and anxiety (Ibrahim and Ehrlich 2020; Liu et al. 2022; Loprinzi et al. 2011; Wang et al. 2021). Unfortunately, there are no specific preventive strategies for chemotherapy-induced peripheral neuropathy (CIPN) (Mezzanotte et al. 2022). In addition, existing treatments are largely palliative and fail to adequately address the underlying pathophysiological mechanisms (Ibrahim and Ehrlich 2020; Loprinzi et al. 2020). Therefore, there is a pressing need to discover new therapies for the prevention and management of CIPN and associated neuropathic pain.

Recent advances in understanding cannabinoid pharmacology have sparked interest in the potential of phytocannabinoids, particularly Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), as therapeutic agents for neuropathic pain (Hansen et al. 2023; Ueberall et al. 2019; Zubcevic et al. 2023). These compounds, derived from the cannabis plant, interact with the endocannabinoid system, involved in modulating pain and inflammation (Maldonado et al. 2016; Woodhams et al. 2017). Notably, THC and CBD have analgesic, anti-inflammatory, and neuroprotective properties, making them attractive candidates for CIPN treatment (D'Andre et al. 2021; Lynch et al. 2014; Nielsen et al. 2022).

In fact, among over 500 different compounds produced by the cannabis plant (Radwan et al. 2021), THC and CBD have been widely studied for the treatment of diverse pathologies of the nervous system (Devinsky et al. 2016; Duncan et al. 2024; Vaney et al. 2004; Woodward et al., 2014). THC, the major psychotropic component of cannabis, has been shown to regulate energy metabolism (Järbe and DiPatrizio 2005), inflammatory processes (Lyman et al. 1989), analgesia (Lichtman and Martin 1991), locomotion (Anderson et al. 1975), and anxiety (Onaivi et al. 1990). The non-psychotropic CBD exhibits anti-inflammatory (Costa et al. 2004), antipsychotic (Zuardi et al. 2006), anxiolytic (Guimarães et al. 1990), and neuroprotective effects (García-Arencibia et al. 2007). Preclinical and clinical studies have reported that the combination of both cannabinoids enhances their therapeutic effects, and it is also proposed that CBD may counteract THC adverse effects (Karniol and Carlini 1973; Klein et al. 2011; Pennypacker and Romero-Sandoval 2020).

In recent decades, research on the therapeutic effects of THC and CBD in pain models has expanded significantly (Comelli et al. 2008; Starowicz and Finn 2017; Xiong et al. 2012). In the context of CIPN, THC and/or CBD have been shown to produce analgesic effects (Harris et al. 2016; King et al. 2017; Ward et al. 2014). However, these effects vary depending on the phytocannabinoid(s) used, dosis, and route of administration, suggesting that their analgesic efficacy may largely depend on the specific THC:CBD ratio and treatment regimen. This variability is reflected in the low efficacy observed in the few studies conducted in CIPN patients, where only mild or no analgesia was reported (Cavaletti et al. 2021; D'Andre et al. 2021; Lynch et al. 2014). This lack of traslation success highlights the importance of thorough preclinical characterization of cannabinoid formulations before advancing to clinical applications.

In addition to peripheral neuropathy and associated pain, other chemotherapy-related adverse effects could be modulated by cannabinoids. However, their role in this modulation has been minimally explored in preclinical models (Blanton et al. 2019; Ostadhadi et al. 2015). Therefore, our study aimed to assess the efficacy of early oral administration of pharmaceutical-grade formulations containing THC and CBD in a rat model of paclitaxel-induced neuropathy. Specifically, we investigated the effects of two distinct THC to CBD ratios (1:1 and 1:20) on various behavioral and physiological parameters associated with neuropathy, including mechanical and thermal sensitivity, locomotor activity, vertical exploratory behaviors, anxiety-related parameters, weight gain, food and water consumption, and liver toxicity.

Materials and methods

Ethics

All the experiments involving animals underwent thorough review and approval by the local Animal Care and Use Committee from Instituto de Investigaciones en Medicina Traslacional (IIMT) CONICET-Universidad Austral, Buenos Aires, Argentina (Assurance number 03/2020 and 04/2020). The design of experimental protocols and behavioral tests adhered to the three Rs principle, aiming to minimize both the quantity of animals used and any discomfort they might experience. These tests strictly followed the

guidelines for animal research outlined by the International Association for the Study of Pain (IASP) and the "Guide for the Care and Use of Laboratory Animals" from the U.S. National Research Council.

Animals and housing

Ninety-six adult male Sprague-Dawley rats, weighing 200 g (6 weeks old), were housed in standard cages with four rats per cage. Animals were obtained from the Animal Care Facility of the School of Biochemistry and Pharmacy at the University of Buenos Aires. Upon arrival at the animal facility of the IIMT CONICET – Universidad Austral, the rats were given at least 7 days to acclimatize to their housing environment. Throughout the acclimatization period and the experimental phase, the rats were maintained in a room with controlled temperature (23 ± 1 °C) and a light cycle of 12 h light/12 hours dark (7 am on, 7 pm off). They had ad libitum access to standard laboratory rodent chow food and tap water, as described in previous studies (Casadei et al. 2021; Miguel et al., 2022; Noya-Riobó et al. 2023).

Drug administration and experimental design

Animals were randomly selected and separated into two cohorts for experimental studies with two different THC:CBD formulations. In each cohort, rats randomly assigned to the different experimental groups were injected with clinically formulated paclitaxel solution (4 mg/ml, Laboratorio Richmond, Argentina) or vehicle (0.9% sterile saline solution) and received clinical grade cannabinoid formulations with known and consistent composition (either THC:CBD 1:1 or THC:CBD 1:20, Cannava SE, Argentina) or vehicle (sesame oil, Cannava SE, Argentina). Thus, 3 experimental groups were included in each experimental study: control (CTL) animals receiving saline solution and sesame oil ($n = 16$); animals receiving paclitaxel (PAX) and sesame oil ($n = 16$); animals treated with PAX plus cannabinoid formulations containing either THC:CBD 1:1 (PAX + THC:CBD 1:1) or THC:CBD 1:20 (PAX + THC:CBD 1:20) ($n = 16$).

PAX was administered intraperitoneally (ip) at a dose of 4 mg/kg/day, with injections given on days 0, 2, 4, and 7, resulting in a cumulative dose of 16 mg/kg (Ullah et al. 2021). Control animals received an equivalent volume of vehicle solution following the same administration protocol. The initiation of PAX/saline administration on day 0 also corresponds with the beginning of daily administration of cannabinoid formulations/sesame oil (Fig. 1). Sesame oil and cannabinoid formulations were orally administered (po) in a volume of 100 µl using an automatic pipette (100 µl/day, THC:CBD 1:1 = 2.5 mg/kg/day THC + 2.5 mg/kg/day CBD or THC:CBD 1:20 = 0.5 mg/kg/day THC + 10 mg/kg/day CBD). The doses of paclitaxel and THC:CBD formulations used are equivalent to those administered to patients (PAX = 135mg/m^2/cycle; THC:CBD 1:1 = 30 mg/day THC and 30 mg/day CBD; THC:CBD 1:20 = 6 mg/day THC and 120 mg/day CBD) (Gurgenci et al. 2024; Inglet et al. 2020; Johnson et al. 2010; Stage et al. 2018).

Before the initiation of the oral treatment, all animals received 100 µl sesame oil to get familiarized to the vehicle solution and the route of administration (Fig. 1). Behavioral and functional experiments were consistently conducted during the light phase as described in subsequent sections. Euthanasia of all animals occurred 14 days after the initiation of the chemotherapy cycle, as illustrated in the timeline diagram. Both paclitaxel administration and behavioral tests were conducted two hours after the administration of cannabinoid formulations, since previous studies indicate that this is when cannabinoid bioavailability is at its peak (Berthold et al. 2023; Hložek et al. 2017).

Mechanical sensitivity: von Frey test

The habituation session to the new environment (d-6) was followed by behavioral assessments conducted before (d-4, baseline measurement), during (d1 and d3), and after (d8 and d12) PAX administration period. Evaluations, performed by a blinded observer between 2 and 6 pm, involved placing animals individually in acrylic chambers on a raised wire mesh platform, allowing them 10 min (min) to acclimate to the new environment (Casadei et al. 2021; Miguel et al.,

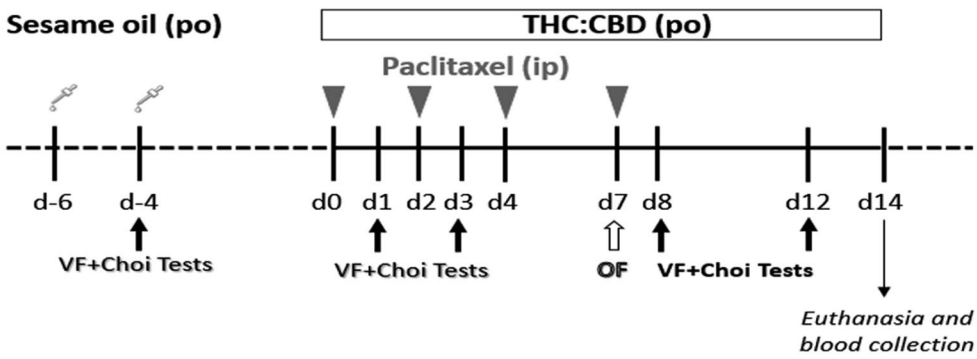

Fig. 1 Experimental design. Schematic representation of the experimental timeline of drug administration, as well as behavioral testing days. d, day; po, oral administration; ip, intraperitoneal; THC:CBD, Δ^9-tetrahydrocannabinol:cannabidiol formulations; VF, von Frey test; OF, open field

2022; Noya-Riobó et al. 2023). To prevent olfactory interference, the acrylic compartments were thoroughly cleaned with 10% ethanol between trials.

During the assessment, rats were alert, not grooming or sleeping and had their four paws in contact with the floor. The evaluation of paw mechanical sensitivity to normally innocuous punctuate and static mechanical stimuli was conducted using a series of eight calibrated von Frey filaments (1.4, 2, 4, 6, 8, 10, 15, 26 g (g), Stoelting, USA). As previously reported (Casadei et al. 2021; Miguel et al., 2022; Noya-Riobó et al. 2023), each filament was manually applied to the mid-plantar surface of each hind paw for 3 s, with at least six measurements performed using the "up-and-down" method (Chaplan et al. 1994; Dixon 1980). Positive responses included robust and immediate paw withdrawals, along with nocifensive behaviors such as repetitive licking or shaking of the paw. Withdrawal responses (g) for both paws were recorded at each testing day. Each paw was treated as an independent measure at each time point.

Subsequently, the von Frey score was calculated, as follows. This composite score was designed to integrate data across the entire experimental period, providing a comprehensive assessment of the decrease in mechanical thresholds. This approach offers a holistic view of the animals' sensory response profile and facilitates comparisons between treatment groups while reducing the influence of day-to-day variability. The withdrawal threshold (g) of each paw at each time point was assigned a score of 2, 1, or 0 points based on whether the threshold fell within the ranges 0–6.99 g, 7–11.99 g, or 12–26 g, respectively. The von Frey score for each paw was calculated as the sum of the scores assigned to the paw at each of the 4 time points (d1, d3, d8 and d12). Thus, the von Frey score had a potential range of 0 to 8 points, with higher scores indicating greater mechanical sensitivity. For example, if a paw was assigned 0 points on d1, 1 point on d3, 2 points on d8 and 2 points on d12, the von Frey score for that paw would be 5. Responses were considered allodynic if paw withdrawal occurred with forces of 7 g or less. Additionally, the development of mechanical allodynia was confirmed if mechanical allodynic responses were detected on at least two independent time points.

Cold sensitivity: Choi test

The animals were tested with the Choi test immediately after performing the von Frey test. Cold sensitivity was measured in the same testing apparatus by applying a drop (100 ml) of acetone to the hind paws, and the response was quantified as paw withdrawal frequency (Choi et al. 1994). Acetone stimulation was performed 5 times on each paw, with a 5-min break between applications to allow the paw´s temperature to return to baseline. The number of brisk paw withdrawals associated with nocifensive behaviors was recorded during the initial 60 s (sec) following acetone application (Miguel et al., 2019; Recalde et al. 2020). Each paw was treated as an independent measure at each time point.

Subsequently, the Choi score was determined. This composite score was designed to integrate data across the entire experimental period, providing a comprehensive assessment of the increase in cold sensitivity. This approach offers a holistic view of the animals' sensory response profile and facilitates comparisons between treatment groups while reducing the influence of day-to-day variability. At each time point (d1, d3, d8 and d12), the number of positive responses was assigned a score of 0, 1, or 2 points, depending on whether the brisk withdrawal count was 0–1, 2, or 3–5, respectively. Therefore, for each paw, the Choi score had a potential range of 0 to 8 points, with higher scores indicating greater thermal sensitivity. For example, if a paw was assigned 1 point on d1, 2 points on d3, 2 points on d8 and 1 point on d12, the cumulative score would be 6. Responses were considered allodynic if paw withdrawals were elicited by 3 or more acetone stimulations. Additionally, the development of cold allodynia in a specific animal was confirmed if cold allodynic responses were detected on at least two independent time points.

Overall health, coat condition and posture

Daily assessments were performed for the evaluation of the coat, with particular attention to any lack of cleanliness or the presence of alopecic plaques, which could indicate deficient or excessive grooming, respectively. Additionally, observations were made regarding evident postural changes and the adoption of antalgic postures, as indirect indicators of spontaneous pain (Miguel et al., 2019; Recalde et al. 2020).

Locomotor activity, vertical exploratory behaviors and anxiety-related parameters: open field test

The open field test was performed on d7, between 1 and 6 pm. Before initiating the test, animals underwent one-hour (h) acclimatization to the experimental conditions of the testing room (Rutten et al. 2014). Each rat was placed in the centre of a black wooden arena measuring 66 × 70 × 43 cm, previously divided into 16 squares using white lines and surrounded by black wooden walls (Parent et al. 2012; Zimcikova et al. 2017). Each animal was recorded during 10 min using a high-quality video camera for subsequent analysis (Soriano et al. 2021). During those 10 min, the experimenter was not present in the room. After the test, each animal was placed in a new cage to avoid disturbing the other animals. The apparatus was thoroughly cleaned

with 10% ethanol between each use to prevent interference from olfactory clues.

Spontaneous locomotor activity was evaluated by measuring total distance covered over a 10-minute period and distance travelled in 2-minute intervals (Dupire et al. 2013; Soriano et al. 2021). Assessment of spontaneous behaviors involved studying rearing movements (animal standing on its hind legs with the front legs in the air), thigmotactic vertical explorations (animal standing on its hind legs with the front legs resting on the wall) and grooming behaviors (animal grooming its face and/or genitals), which were assessed during the 10-min period (Rutten et al. 2014; Soriano et al. 2021; Zimcikova et al. 2017). Total number of episodes of vertical exploration, including rearing and thigmotactic movements, was calculated.

Anxiety-like behaviors were assessed during the initial 5 min in the open field device by analysing various parameters. These included the latency to exit the central area for the first time, the percentage of time each rat spent in the central area (four central squares), and the number of entries into the central zone (Long et al. 2010; Parent et al. 2012; Soriano et al. 2021).

Weight gain, food and water intake

Body weights were recorded three times a week throughout the entire experimental period (Miguel et al., 2019; Recalde et al. 2020). The percentage increase in weight for each group at every time point was also computed using the formula: [(current mean weight - starting mean weight) / starting mean weight] x 100.

Daily recordings of food consumption and water consumption were conducted in the morning, both during PAX administration (d1 and d2) and post-administration (d10, d11, and d12) periods. Food and water intakes were assessed at the cage level and normalized to the total body weight (g) of the four animals in each cage (Laaksonen et al. 2013). Intakes were adjusted for body weight as follows: food intake was calculated by dividing the grams of food consumed per cage by the total body weight (g) of all animals in the cage. Similarly, water intake was determined by dividing the milliliters of water consumed per cage by the total body weight (g) of the animals in the cage.

Liver enzymes: serum analysis

At the end of each experiment, animals were placed in a closed chamber for induction of anaesthesia using isoflurane (Piramal, Pennsylvania, USA) and then immediately euthanized by decapitation (Noya-Riobó et al. 2023). Five ml of blood were collected and then kept at room temperature for 10 min. Samples were centrifuged at 3000 rpm for 10 min. Serum obtained was used to determine the levels of the liver enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Enzyme activity was determined using the Alinity AST2 Aspartate Amino Reagent kit (Abbott Laboratories, Cat. No. 04T86) and the Alinity ALT2 Alanine Amino Reagent kit (Abbott Laboratories, Cat. No. 04T84). Levels of activity were expressed as units per liter (U/l).

Statistical analysis

Data was analysed using GraphPad Prism 8 (GraphPad Software Inc., La Jolla, CA, USA). All the results were expressed as mean ± standard error of the mean (SEM). Parameters evaluated along time (distance travelled in 2-min intervals, weight, weight increase, food and water intakes) were analysed initially using repeated measures analysis of variance (two-way ANOVA) with two factors: treatment (between-subjects factor) and time (within-subjects factor). If the two-way ANOVA revealed a significant interaction between these factors, additional analyses were performed to examine the main effect of each factor by using one-way ANOVA and unpaired Student's t test t (for normally distributed data) or Kruskal–Wallis tests (for non-normally distributed data). On the other hand, the effect of treatment on parameters that did not depend on time (von Frey and Choi scores, total distance travelled, episodes of vertical activity, anxiety-related behaviors and liver enzyme activities) was analysed using one-way ANOVA or Kruskal–Wallis tests, as appropriate. Multiple comparisons were performed using Bonferroni's test (ANOVA) and Dunn's pairwise test (Kruskal–Wallis). P values < 0.05 were considered statistically significant.

Results

THC:CBD formulations prevented the development of mechanical and cold hypersensitivity and allodynia in rats treated with paclitaxel

Rats subjected to PAX treatment exhibited mechanical and thermal hypersensitivity, as indicated by a notable increase in von Frey scores (THC:CBD 1:1 Experiment: $H = 21.0$, $p < 0.0001$, Kruskal–Wallis test, mean rank difference of CTL vs. PAX= -27.4, Dunn's test; THC:CBD 1:20 Experiment: $H = 8.5$, $p = 0.014$ Kruskal–Wallis test, mean rank difference of CTL vs. PAX= -13.8, Dunn's test; Fig. 2A and B) and Choi scores (THC:CBD 1:1 Experiment: $H = 21.4$, $p < 0.0001$, Kruskal–Wallis test, mean rank difference of CTL vs. PAX= -26.5, Dunn's test; THC:CBD 1:20 Experiment: $H = 8.8$, $p = 0.012$, Kruskal–Wallis test, mean rank difference of CTL vs. PAX= -15.6, Dunn's test; Fig. 2C

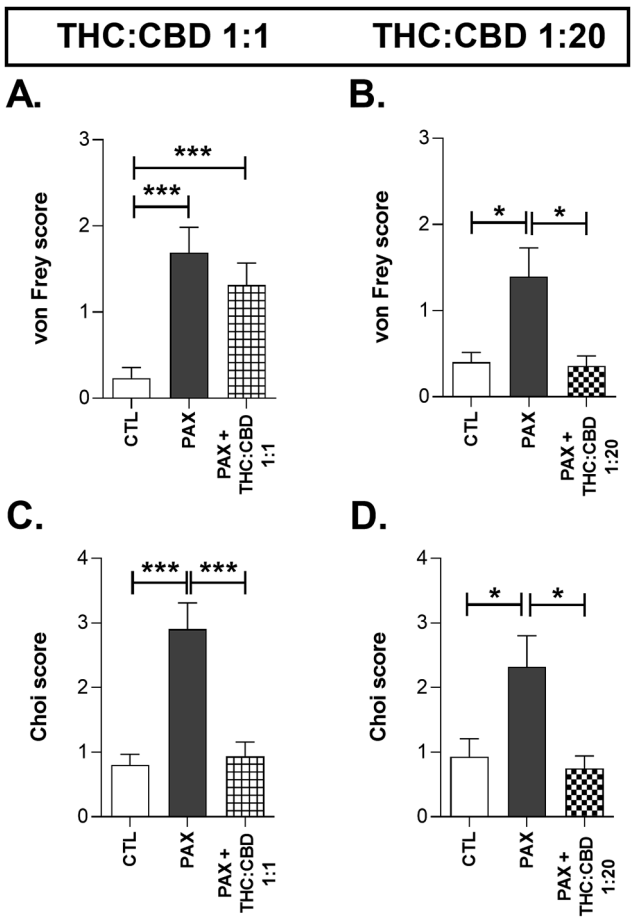

Fig. 2 Mechanical and cold sensitivity. **A-B.** von Frey scores. **C-D.** Choi scores. Data shown represent the mean ± SEM. $n = 28–32$ independent measures per experimental group. Parameters were analyzed by Kruskal-Wallis test and Dunn's post-hoc comparison. *$p < 0.05$, ***$p < 0.001$

and D), respectively. Early and sustained administration of a THC:CBD 1:1 formulation was able to remarkably prevent PAX-induced increase in Choi score (H = 21.4, $p < 0.0001$, Kruskal–Wallis test, mean rank difference of PAX vs. PAX + THC:CBD 1:1 = 25.5, Dunn's test), thereby preventing thermal (cold) hypersensitivity (Fig. 2C). However, von Frey score, indicative of mechanical sensitivity, remained elevated in this group of animals (H = 21.0, $p < 0.0001$, Kruskal–Wallis test, mean rank difference of CTL vs. PAX + THC:CBD 1:1 = -23,4, Dunn's test; Fig. 2A). On the other hand, administration of a THC:CBD 1:20 formulation effectively prevented both mechanical (H = 8.5, $p = 0.014$, Kruskal–Wallis test, mean rank difference of PAX vs. PAX + THC:CBD 1:20 = 15.4, Dunn's test; Fig. 2B), and thermal hypersensitivities (H = 8.8, $p = 0.012$, Kruskal–Wallis test, mean rank difference of PAX vs. PAX + THC:CBD 1:20 = 16.5, Dunn's test; Fig. 2D). Notably, both formulations reduced the Choi score by nearly 70% compared to the PAX group (Fig. 2C and D).

Mechanical and thermal allodynic responses ocurred in 17% and 36% of PAX-treated animals, respectively. Interestingly, only 6% of animals receiving either the THC:CBD 1:1 or 1:20 developed mechanical allodynia. Moreover, while 19% of animals treated with THC:CBD 1:1 presented thermal allodynic responses, such behavior was absent in those treated with THC:CBD 1:20.

In sum, both combinations of THC and CBD prevented paclitaxel-induced cold hypersensitivity while only THC:CBD 1:20 could prevent mechanical hypersensitivity. In addition, both formulations reduced the number of animals experiencing mechanical or thermal allodynia.

Neither paclitaxel nor THC:CBD formulations had any effect on coat appearance or body posture

None of the animals under evaluation displayed observable piloerection or evident weakness of the hind limbs throughout the experimental timeframe. Moreover, there were no alterations in body posture, including limb retraction or protective behaviors. Furthermore, there were no observable changes in the physical appearance of the animals, such as a diminished coat cleanliness or the presence of alopecic plaques. These indicators, which could suggest inadequate or excessive grooming respectively, were absent across all the experimental groups over the entire experimental period.

Cannabinoids could not alleviate paclitaxel-induced hypo-locomotion, but did not cause any additional motor impairment

PAX-treated animals showed reduced spontaneous locomotor activity, indicated by a shorter distance travelled in the open field arena during the 10-minute period compared to CTL group (THC:CBD 1:1 Experiment: $F_{(2,36)} = 3.6$, $p = 0.037$, one-way ANOVA; $p < 0.05$, Bonferroni *post hoc*; THC:CBD 1:20 Experiment: $F_{(2,38)} = 7.4$, $p = 0.002$, one-way ANOVA; $p < 0.01$, Bonferroni *post hoc*; Fig. 3A and B). Decreased exploratory activity was also observed in PAX + THC:CBD 1:20 group ($p < 0.05$ vs. CTL, Fig. 3A) but not in rats treated with THC:CBD 1:1 ($p > 0.05$ vs. CTL, Fig. 3B). However, no significant differences were observed between animals receiving paclitaxel alone and those receiving paclitaxel plus cannabinoids (THC:CBD 1:1 and THC:CBD 1:20 Experiments: $p > 0.05$ in both cases; Fig. 3A and B).

For the analysis of cumulative distance travelled in 2-min intervals, two-way ANOVA revealed a significant interaction between treatment and time intervals (THC:CBD 1:1 Experiment: $F_{(8,\ 144)} = 2.7$, $p = 0.009$, two-way ANOVA; THC:CBD 1:20 Experiment: $F_{(8,\ 152)} = 4.2$, $p < 0.001$, two-way ANOVA).Therefore, additional analyses were

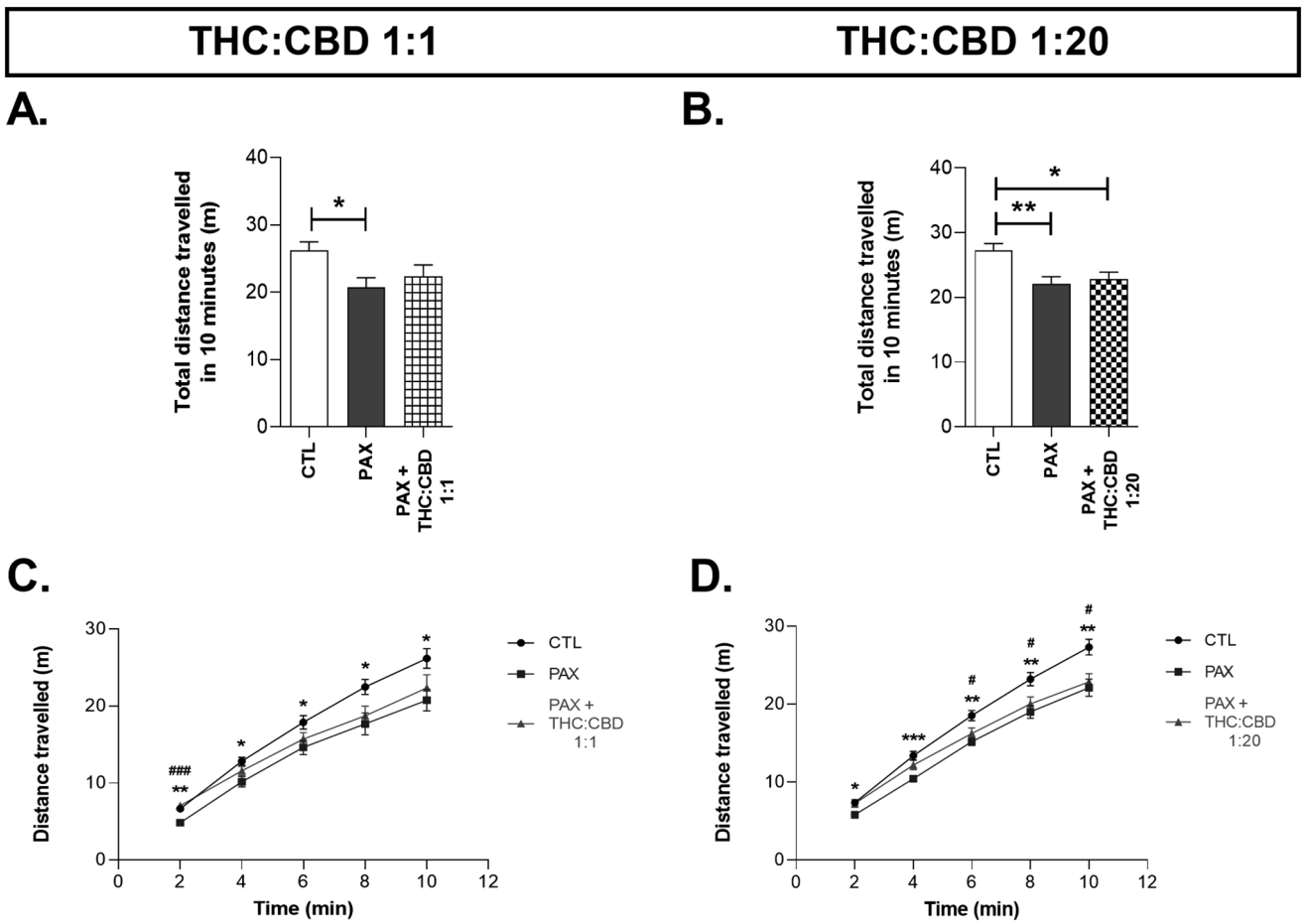

Fig. 3 Spontaneous locomotor activity. **A-B.** Total distance travelled in 10 min. **C-D.** Distance travelled in 2-minute intervals. Data shown represent the mean ± SEM. $n = 12–15$ animals per experimental group. Parameters were analysed by one-way ANOVA and Bonferroni post-test. *Significant differences between CTL and PAX groups. # Significant differences between PAX and PAX + THC:CBD groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$

performed to examine the main effect of treatment in the different time intervals by using one-way ANOVA and Bonferroni post test. Animals in PAX group once again exhibited decreased exploratory activity across time intervals (THC:CBD 1:1 Experiment: $p < 0.01$ in minute 2 and $p < 0.05$ in minutes 4–10; THC:CBD 1:20 Experiment: $p < 0.05$ in minute 2, $p < 0.001$ in minutes 4 and $p < 0.01$ in minutes 6–10; Fig. 3C and D). In the case of animals receiving PAX + THC:CBD a reduced distance travelled was observed only in specific time frames (THC:CBD 1:1 Experiment: $p < 0.001$ in minute 2; THC:CBD 1:20 Experiment: $p < 0.05$ in minutes 6–10; Fig. 3C and D). On the other hand, cannabinoids produced a similar reduction in activity across time intervals compared to the PAX-group (THC:CBD 1:1 and THC:CBD 1:20 Experiments: $p > 0.05$ in all times; Fig. 3A and B).

Thus, the analysis of the distance travelled by the different experimental groups revealed no effects of cannabinoid formulations on PAX-induced decrease in locomotor activity.

THC:CBD 1:1 restored rearing behavior after paclitaxel administration

The number of rearing episodes was significantly reduced in animals receiving PAX compared to CTL group (THC:CBD 1:1 Experiment: $F_{(2,39)} = 8.7$, $p < 0.001$, one-way ANOVA; $p < 0.001$, Bonferroni *post hoc*; THC:CBD 1:20 Experiment: $F_{(2,37)} = 3.7$, $p = 0.034$, one-way ANOVA; $p < 0.05$, Bonferroni *post hoc*; Fig. 4A and B). In contrast, PAX + THC:CBD groups showed no differences when compared to CTL animals (THC:CBD 1:1 and THC:CBD 1:20 Experiments: $p < 0.05$; Fig. 4A and B). However, only animals receiving 1:1 THC:CBD displayed a significantly higher number of rearing movements when compared to PAX-treated animals (THC:CBD 1:1 Experiment: $p < 0.05$. THC:CBD 1:20 Experiment: $p > 0.05$; Fig. 4A and B).

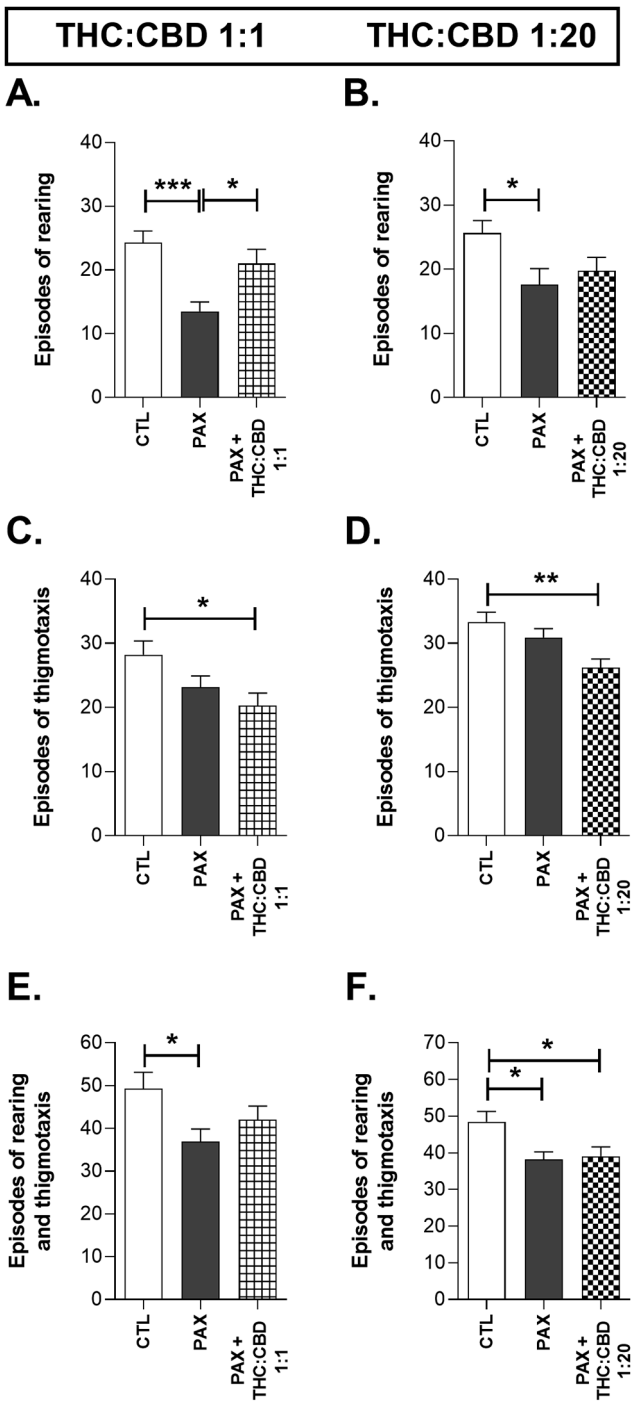

Fig. 4 Spontaneous vertical activity. **A-B.** Number of episodes of rearing movements. **C-D.** Number of episodes of thigmotaxis. **E-F.** Total number of episodes of rearing and thigmotaxis. Data shown represent the mean ± SEM. $n = 13–15$ animals per experimental group. Parameters were analysed by one-way ANOVA and Bonferroni post-test. *$p<0.05$, **$p<0.01$, ***$p<0.001$

Thigmotaxis behaviour was reduced in animals receiving both paclitaxel and THC:CBD formulations (THC:CBD 1:1 Experiment: $F_{(2,40)}=4.2$, $p=0.022$, one-way ANOVA; $p<0.05$, Bonferroni *post hoc*; THC:CBD 1:20 Experiment: $F_{(2,40)}=6.5$, $p=0.004$, one-way ANOVA; $p<0.01$, Bonferroni *post hoc*; Fig. 4C and D). A slight reduction not reaching statistical significance was observed in animals receiving only PAX (THC:CBD 1:1 and THC:CBD 1:20 Experiments: $p<0.05$; Fig. 4C and D).

In addition, vertical exploratory activity, evaluated as the total number of rearing and thigmotactic movements, was significantly reduced in PAX group (THC:CBD 1:1 Experiment: $F_{(2,40)}=4.2$, $p=0.022$, one-way ANOVA; $p<0.05$, Bonferroni *post hoc*; THC:CBD 1:20 Experiment: $F_{(2,40)}=6.5$, $p=0.004$, one-way ANOVA; $p<0.01$, Bonferroni *post hoc*; Fig. 4E and F) and in PAX-treated animals receiving 1:20 THC:CBD (THC:CBD 1:1 Experiment: $p>0.05$; THC:CBD 1:20 Experiment: $p<0.05$; Fig. 4E and F).

Grooming behaviour was not affected by paclitaxel treatment (THC:CBD 1:1 Experiment: $F_{(2,39)}=1.5$, $p=0.246$, one-way ANOVA; THC:CBD 1:20 Experiment: $F_{(2,37)}=6.2$, $p=0.005$, one-way ANOVA; $p>0.05$, Bonferroni *post hoc*; data not shown). However, THC:CBD 1:20 produced an increase in the number of grooming episodes when compared to CTL ($p<0.01$) and PAX-treated ($p<0.05$) animals (data not shown).

Taken together, these results demonstrate that the reduction in rearing behavior induced by paclitaxel was attenuated by treatment with THC:CBD 1:1. Additionally, cannabinoid preparations accentuate the tendency towards decreased episodes of thigmotaxis observed in PAX-group. However, this effect on thigmotaxis behavior did not modify PAX-induced decrease in vertical exploratory activity. Finally, the formulation containing THC:CBD 1:20 increased grooming behaviour in animals treated with paclitaxel.

Anxious-like behaviors in rats treated with PAX were neither alleviated nor exacerbated by cannabinoids

PAX-treated animals spent less time in the central area (THC:CBD 1:1 Experiment: $F_{(2,42)}=4.8$, $p=0.014$, one-way ANOVA; $p<0.05$, Bonferroni *post hoc*; THC:CBD 1:20 Experiment: $F_{(2,40)}=3.7$, $p=0.035$, one-way ANOVA; $p<0.05$, Bonferroni *post hoc*; Fig. 5C and D) and entered the central area fewer times compared to the CTL group (THC:CBD 1:1 Experiment: $F_{(2,41)}=1.5$, $p=0.242$, one-way ANOVA; THC:CBD 1:20 Experiment: $F_{(2,41)}=5.0$, $p=0.011$, one-way ANOVA; $p<0.01$, Bonferroni *post hoc*; Fig. 5E and F). However, the latency to leave the central area was not altered by paclitaxel (THC:CBD 1:1 Experiment: $H=5.9$, $p=0.050$, Kruskal–Wallis test; THC:CBD 1:20 Experiment: $H=0.5$, $p=0.783$ Kruskal–Wallis test; Fig. 5A and B). Treatment with THC:CBD formulations did not affect either the latency or the number of central area entries compared to CTL- or PAX-groups (THC:CBD

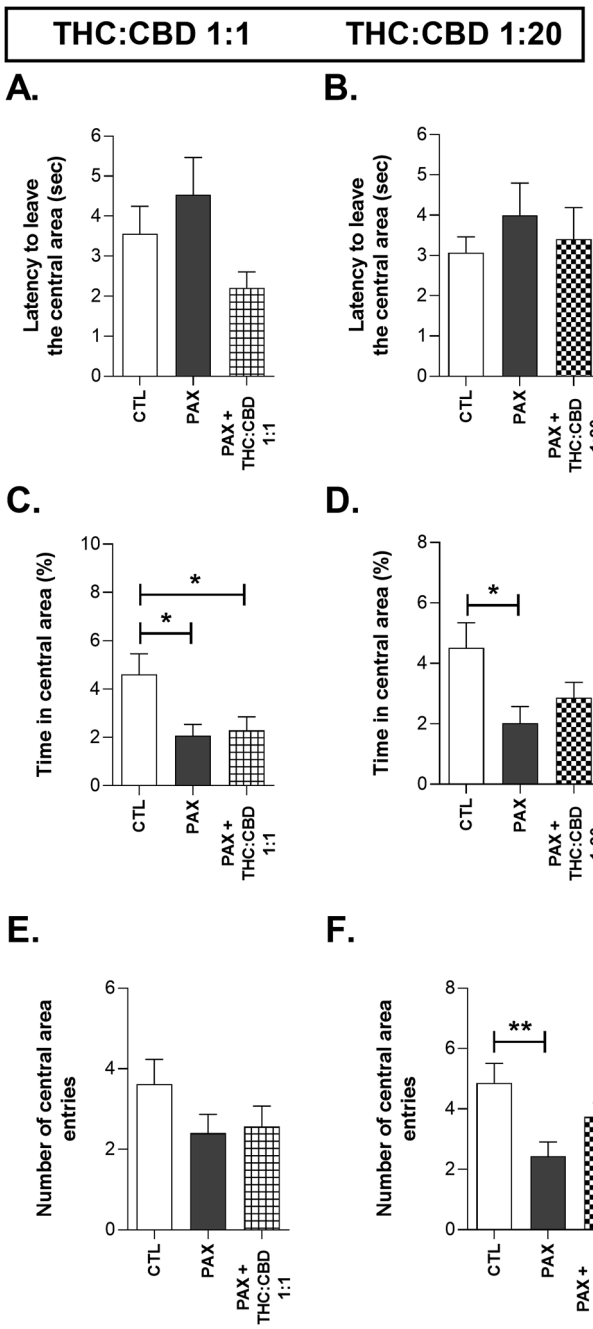

Fig. 5 Anxiety-like behaviour. **A-B.** Latency to exit the central area. **C-D.** Percentage of time spent in the central area. **E-F.** Number of entries into the central zone. Data shown represent the mean ± SEM. $n = 13–16$ animals per experimental group. Parameters were analysed by one-way ANOVA and Bonferroni post-test, or Kruskal-Wallis test and Dunn's post-hoc comparison as appropriate. *$p < 0.05$

1:1 and THC:CBD 1:20 Experiments: $p > 0.05$ in both parameters; Fig. 5A, B, E and F). Only animals receiving THC:CBD 1:1 showed a significant reduction in time spent in the central area ($p < 0.05$ vs. CTL; Fig. 5C).

These results indicate that THC:CBD formulations did not alleviate nor exacerbate the anxious-like phenotype induced by paclitaxel.

THC:CBD formulations did not modify the progressive attenuation in weight gain observed in rats treated with paclitaxel

A significant interaction between treatment and time was observed for body weight (THC:CBD 1:1 Experiment: $F_{(16, 344)} = 14.1$, $p < 0.0001$, two-way ANOVA; THC:CBD 1:20 Experiment: $F_{(16, 328)} = 12.3$, $p < 0.0001$, two-way ANOVA) and weight gain (THC:CBD 1:1 Experiment: $F_{(16, 344)} = 12.5$, $p < 0.0001$, two-way ANOVA; THC:CBD 1:20 Experiment: $F_{(16, 344)} = 9.1$, $p < 0.0001$, two-way ANOVA). Therefore, an analysis of simple effects of treatment in different experimental days was conducted. When compared to CTL animals, lower body weight was observed in PAX-treated animals from day 7 (THC:CBD 1:1 Experiment: $p < 0.05$ on days 7 and 13 and $p < 0.01$ on days 9 and 11; THC:CBD 1:20 Experiment: $p < 0.05$ on day 13, $p < 0.01$ on days 7, 9 and 11; one-way ANOVA and Bonferroni post-test for all date; Fig. 6A and B). Similarly, an attenuation in weight gain was observed in PAX-group compared to CTL-group (THC:CBD 1:1 Experiment: $p < 0.01$ on days 4, 7 and 13 and $p < 0.001$ on days 9 and 11, one-way ANOVA and Bonferroni post-test; THC:CBD 1:20 Experiment: $p < 0.05$ on days 7 and 13, $p < 0.01$ on day 11, $p < 0.001$ on day 9, Kruskal–Wallis test and Dunn's test; Fig. 6C and D). The body weight of animals treated with both paclitaxel and cannabinoids was similar to that of animals treated with paclitaxel alone, but significantly lower than that of CTL animals (THC:CBD 1:1 Experiment: $p < 0.01$ on days 7, 11 and 13 and $p < 0.001$ on day 9; THC:CBD 1:20 Experiment: $p < 0.05$ on day 13, $p < 0.01$ on days 7, 9 and 11; Fig. 6A and B). Also, an attenuation in weight gain was detected in animals treated with both cannabinoid formulations (THC:CBD 1:1 Experiment: $p < 0.001$ on days 4, 7, 9, 11 and 13; THC:CBD 1:20 Experiment: $p < 0.05$ on days 7 and 13, $p < 0.01$ on day 11, $p < 0.001$ on day 9; Fig. 6C and D) compared to CTL group. However, changes in body weight and gain weight were similar when comparing PAX-treated animals with those receiving PAX + THC:CBD formulations.

Thus, the results revealed no effects of cannabinoid formulations on PAX-induced attenuation in body weight and weight gain.

Chan s in paclitaxel-treated rats were not modulated by cannabinoids

When analysing food and water intakes over time, a significant interaction between treatment and time was

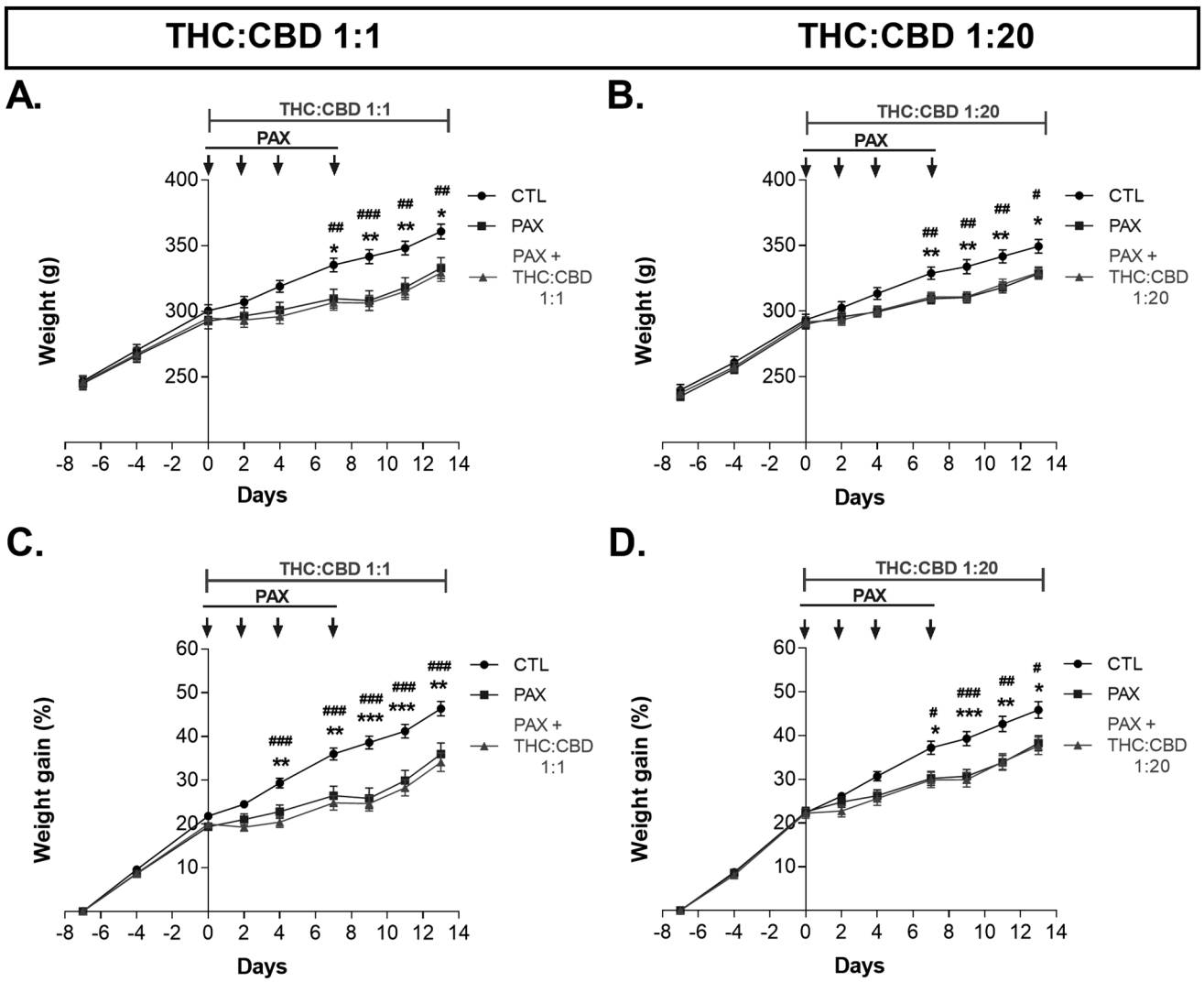

Fig. 6 Body weight throughout the experimental period. **A-B.** Weight. **C-D.** Weight gain. Data shown represent the mean ± SEM. $n = 13–16$ animals per experimental group. Parameters were analysed by one-way ANOVA and Bonferroni post-test, or Kruskal-Wallis test and Dunn's post-hoc comparison as appropriate. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ represent significant differences between CTL and PAX groups, while $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ represent significant differences between PAX and PAX + THC:CBD groups

observed (THC:CBD 1:1 Experiment: $F_{(2,\ 6)}=10.4$, $p=0.011$, two-way ANOVA; THC:CBD 1:20 Experiment: $F_{(2,\ 9)}=24.4$, $p<0.0001$, two-way ANOVA; data correspond to food intake. THC:CBD 1:1 Experiment: $F_{(2,\ 18)}=14.1$, $p=0.006$, two-way ANOVA; THC:CBD 1:20 Experiment: $F_{(2,\ 8)}=18.9$, $p<0.001$, two-way ANOVA; data correspond to water intake). Additional analyses were performed to examine the main effect of treatment in the different time intervals, as well as the specific effect of time in each experimental group by using one-way ANOVA and Bonferroni post-test and paired t-test, respectively.

In PAX-treated animals, food intake was reduced during PAX administration (THC:CBD 1:1 Experiment: $F_{(2,3)}=7.9$, $p=0.063$, one-way ANOVA; THC:CBD 1:20 Experiment: $F_{(2,9)}=12.3$, $p=0.003$, one-way ANOVA; $p<0.01$, Bonferroni *post hoc*; Fig. 7A and B), while it was increased in the late phase, compared to CTL group (THC:CBD 1:1 Experiment: $F_{(2,3)}=8.2$, $p=0.061$, one-way ANOVA; THC:CBD 1:20 Experiment: $F_{(2,9)}=19.6$, $p=0.0005$, one-way ANOVA; $p<0.01$, Bonferroni *post hoc*; Fig. 7A and B). Similar changes in food intake during and after PAX administration period were observed in animals receiving paclitaxel and THC:CBD 1:1 ($p<0.01$ for early phase; $p<0.001$ for later phase). In CTL animals, food intake was higher in the initial phase than at later time points (THC:CBD 1:1 Experiment: $t_{(1)}=31.0$, $p=0.021$, paired t-test; THC:CBD 1:20 Experiment: $t_{(3)}=36.7$, $p<0.0001$, paired t-test; Fig. 7A and B).

In the case of water intake, paclitaxel alone or in combination with THC:CBD formulations induced a reduction in

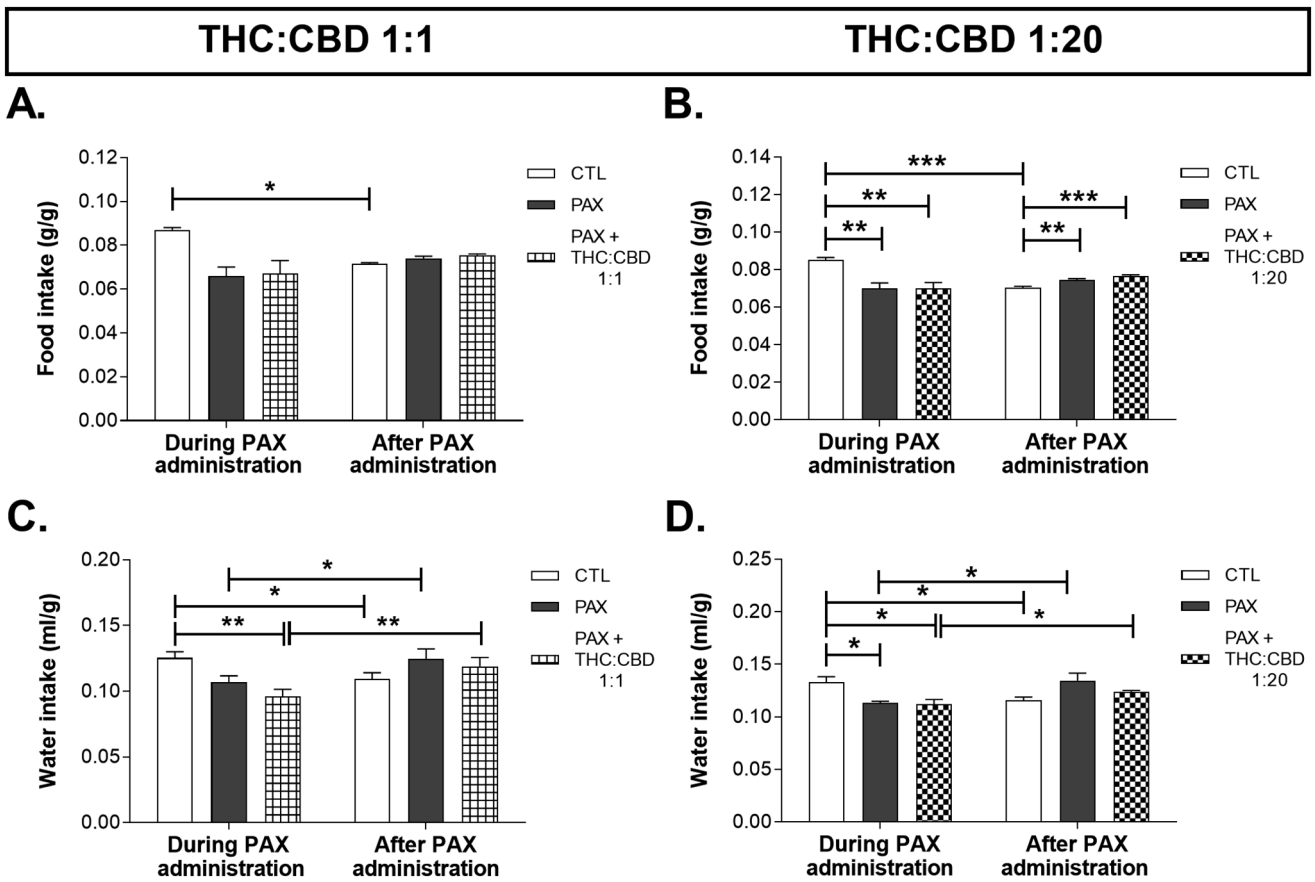

Fig. 7 Feeding behaviour. **A-B.** Food intake. **C-D.** Water intake. Food and water consumptions evaluated during (d1-d2) and after (d10-d12) the period of PAX administration. Data shown represent the mean ± SEM. $n = 2–4$ cages per experimental group. Parameters were analysed by one-way ANOVA and Bonferroni post-test, or paired t-test as appropriate. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

water consumption during PAX-administration period compared to CTL group (THC:CBD 1:1 Experiment: $F_{(2,9)} = 8.7$, $p = 0.008$, one-way ANOVA; $p < 0.01$ to PAX + THC:CBD, Bonferroni *post hoc*; THC:CBD 1:20 Experiment: $F_{(2,8)} = 8.4$, $p = 0.011$, one-way ANOVA; $p < 0.05$ to PAX and PAX + THC:CBD, Bonferroni *post hoc*; Fig. 7C and D). When comparing the initial period of PAX administration with the post-chemotherapy phase, CTL group showed decreased water intake (THC:CBD 1:1 Experiment: $t_{(3)} = 3.9$, $p = 0.029$, paired t-test; THC:CBD 1:20 Experiment: $t_{(2)} = 7.4$, $p = 0.018$, paired t-test; Fig. 7C and D), while PAX (THC:CBD 1:1 Experiment: $t_{(3)} = 3.4$, $p = 0.043$, paired t-test; THC:CBD 1:20 Experiment: $t_{(3)} = 3.8$, $p = 0.032$, paired t-test; Fig. 7C and D) and PAX + THC:CBD groups increased consumption (THC:CBD 1:1 Experiment: $t_{(3)} = 6.6$, $p = 0.007$, paired t-test; THC:CBD 1:20 Experiment: $t_{(3)} = 3.3$, $p = 0.044$, paired t-test; Fig. 7C and D) at later time points.

In sum, the administration of cannabinoids did not interfere with the changes in food or water intakes induced by paclitaxel.

Paclitaxel and THC:CBD formulations did not modify serum transaminase levels

The activity levels of serum AST (THC:CBD 1:1 Experiment: $F_{(2,16)} = 2.3$, $p = 0.129$, one-way ANOVA; THC:CBD 1:20 Experiment: $F_{(2,16)} = 2.2$, $p = 0.144$, one-way ANOVA; Fig. 8A and B) and ALT (THC:CBD 1:1 Experiment: $F_{(2,17)} = 2.3$, $p = 0.127$, one-way ANOVA; THC:CBD 1:20 Experiment: $F_{(2,15)} = 1.1$, $p = 0.371$, one-way ANOVA; Fig. 8C and D) were similar between experimental groups. Thus, neither paclitaxel nor cannabinoids affected serum transaminase levels.

Discussion

Paclitaxel is a commonly used antitumor agent in the first-line treatment of various cancers, such as lung, breast and ovarian cancer (Anand et al. 2023). The development of peripheral neuropathy and neuropathic pain is one of the main factors limiting dosing and, therefore, efficacy of paclitaxel-based chemotherapy (da Costa et al. 2020). In

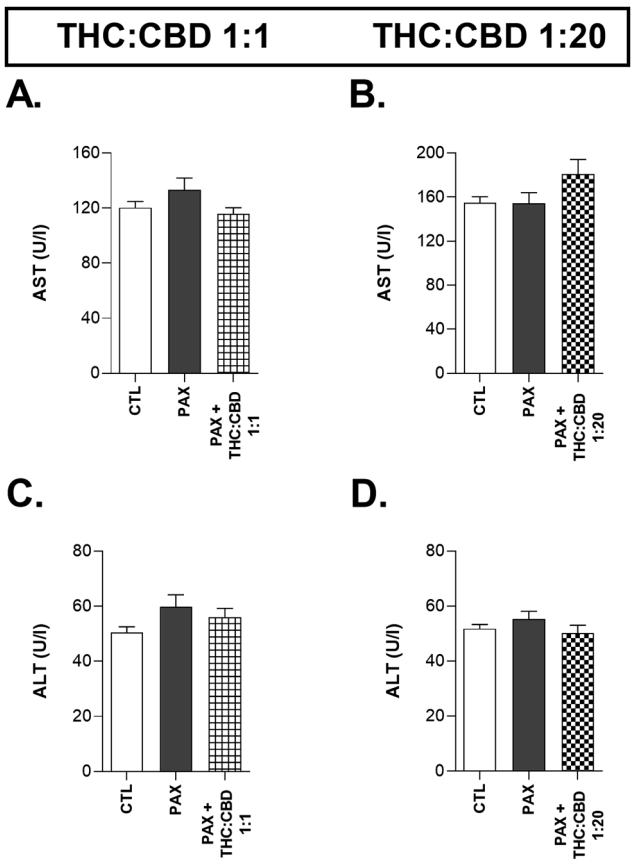

Fig. 8 Liver enzyme activities in serum. **A-B.** Aspartate amino transferase (AST) activity. **C-D.** Alanine amino transferase (ALT) activity. Data shown represent the mean ± SEM. $n = 5–8$ animals per experimental group. Parameters were analysed by one-way ANOVA and Bonferroni post-test

recent years, the use of cannabinoids has increased among patients with chronic pain; however, there is insufficient evidence to support their use for specific conditions (MacCallum and Russo 2018).

In this study, we assessed the effects of oral formulations with varying THC-to-CBD ratios on paclitaxel-induced pain and other chemotherapy-related adverse effects, including alterations in locomotion, spontaneous activity, feeding, water intake, anxiety-related behavior, and liver toxicity. THC:CBD 1:1 formulation was included for its balanced combination often associated with synergistic therapeutic effects (Hardy et al. 2024; Johnson et al. 2010), while the THC:CBD 1:20 formulation was selected to emphasize CBD and assess its efficacy in mitigating THC-associated adverse effects (Freeman et al. 2019; Pennypacker and Romero-Sandoval 2020). Our findings show that these formulations differ in their ability to prevent paclitaxel-induced pain-related behaviors and reductions in spontaneous activity. Importantly, these formulations do not exacerbate other paclitaxel-induced adverse effects, such as hypo-locomotion or anxiety-like behaviors. Given that the administration route and doses were comparable to those used in approved cannabinoid-based medications (Inglet et al. 2020), these findings suggest potential therapeutic applications not only for managing pain but also for enhancing daily activities in patients undergoing paclitaxel treatment.

Sensory abnormalities predominantly characterize paclitaxel-induced neuropathy, and persistent pain is generally the most severe symptom (da Costa et al. 2020; Ibrahim and Ehrlich 2020). Patients normally report heightened sensitivity to touch, pressure, mildly cold or warm temperatures. As previously demonstrated, experimental animals treated with paclitaxel also develop induced pain-like behaviors (Bacalhau et al. 2023). In both patients and rats, hypersensitivity develops dynamically, following distinct temporal patterns of onset and progression. Therefore, composite scores that integrate data over time and provide a holistic view of the animals' sensory response profiles facilitate treatment group comparisons while minimizing the impact of day-to-day variability. In our study, we found that early oral administration of both THC:CBD formulations prevented paclitaxel-induced cold allodynia. However, only THC:CBD 1:20 effectively prevented hypersensitivity induced by mechanical stimuli. This differential efficacy suggests a dose-dependent effect, where a lower THC dose combined with a higher CBD dose more effectively blocks the development of heightened mechanical sensitivity.

Consistent with these findings, other experimental models of neuropathic pain have shown that increasing the CBD-to-THC ratio enhances the analgesic effects on mechanical allodynia in a synergistic manner (Comelli et al. 2008; Mitchell et al. 2021). Interestingly, synergistic effects on paclitaxel-induced mechanical hypersensitivity have also been reported when combining very low, individually ineffective doses of CBD and THC (King et al. 2017). Although no changes in body posture or coat condition were observed after paclitaxel administration, either alone or in combination with THC and CBD, the presence of ongoing spontaneous pain cannot be fully ruled out. Overall, our findings offer two potential therapeutic options that could address the most severe symptoms in patients developing paclitaxel-induced neuropathy.

The decline in daily activities frequently reported by cancer patients experiencing paclitaxel-induced neuropathy reflects the profound impact of this treatment-related side effect (Kurt et al. 2023; Srivastava et al. 2022). In addition, patients receiving cannabinoid-based therapies have also reported a decrease in overall activity (Inglet et al. 2020). Therefore, we evaluated the effects of both paclitaxel and cannabinoids on horizontal (locomotion) and vertical (rearing plus thigmotaxis) exploratory activities.

Previous studies have shown that THC can induce hypo-locomotion in naïve animals through the activation of CB1

receptor (Metna-Laurent et al. 2017). This effect may be enhanced or remain unchanged with the co-administration of CBD (Calapai et al. 2022). However, in the context of paclitaxel-induced neuropathy, the lack of changes in locomotion following the administration of THC and CBD suggests the potential safety of the evaluated formulations.

Rearing is a natural behavior in rodents that enables them to collect visual, olfactory, and auditory cues from their surroundings, while thigmotaxis, a variation of rearing where the front paws contact a surface for support, offers additional somatosensory input (Lever et al. 2006). Spontaneous vertical exploratory activity, involving both rearing and thigmotactic behaviors, has been poorly studied in preclinical models using cannabinoids. In our study, a significant reduction in total vertical exploratory activity was observed in paclitaxel-treated rats, which was unaffected by cannabinoid administration. In contrast, co-administration of equal amounts of THC and CBD mitigated the reduction in rearing activity induced by paclitaxel. However, thigmotaxis behavior decreased in animals receiving THC:CBD 1:1 or 1:20. Nonetheless, neither effect positively nor negatively influenced the overall reduction in total vertical activity caused by paclitaxel.

In this regard, Hlozek and colleagues previously reported that neither THC nor CBD significantly altered thigmotaxis behavior; however, their combined administration did reduce thigmotaxis in naïve animals (Hložek et al. 2017). In contrast, rearing behavior appears unaffected in animals receiving CBD (Long et al. 2010; Espejo-Porras et al. 2013), while it is diminished by the administration of vaporized cannabis or THC (Bruijnzeel et al. 2016; Järbe et al. 2002). Our results indicate that paclitaxel administration led to a decrease in rearing behavior, suggesting a reduction in daily activities. Notably, this reduction was prevented by the administration of equal amounts of THC and CBD.

Patients undergoing paclitaxel treatment may also experience cognitive deficits along with mood disorders, including depression and anxiety (da Costa et al. 2020). Preclinical studies further indicate that paclitaxel-induced neuropathy can lead to alterations in affective behaviors (Toma et al. 2017). Additionally, both THC and CBD have demonstrated the ability to modulate anxiety in clinical and animal studies (Bahji et al. 2020; Hasbi et al. 2023). In our study, animals treated with paclitaxel, either alone or in combination with THC:CBD, exhibited increased anxiety-like behaviors, as evidenced by making fewer entries and spending less time in the central area. Notably, these anxious-like behaviors induced by paclitaxel were unaffected by cannabinoid formulations, suggesting that neither the anxiolytic nor anxiogenic effect of THC, nor the anxiolytic effect of CBD (Henson et al. 2022) were evident in this context.

Previous preclinical and clinical research has shown that paclitaxel can induce changes in weight and body mass (Hess et al. 2007; Ray et al. 2011), a side effect often linked with reduced appetite (Kaizu et al. 2021). THC and CBD have opposing effects on appetite regulation, with THC stimulating food intake and CBD reducing it (Ligresti et al. 2016; Spanagel and Bilbao 2021). In this study, we observed that paclitaxel-induced attenuation in weight gain persisted despite the administration of THC:CBD. Consistently, food and water intake decreased only during the initial phase, corresponding to paclitaxel administration period, in both paclitaxel-only and THC:CBD groups. Notably, previous rodent studies have demonstrated a strong interrelationship between food and water intake, and their link to weight gain (Bachmanov et al. 2002; Minematsu et al. 1994). Interestingly, clinical studies across various conditions present mixed findings: while some report increased appetite and weight gain with cannabinoid use, others find no such effects, whether THC and CBD are used alone or in combination (Inglet et al. 2020; Spanagel and Bilbao 2021).

Hepatotoxicity is a rare adverse effect of paclitaxel treatment (Doğan and Gökhan 2024). However, cannabinoids, particularly CBD, can lead to elevated hepatic transaminase enzymes (Anciones and Gil-Nagel 2020; dos Santos et al. 2021). In our neuropathic pain model, we observed no changes in serum AST and ALT levels in animals treated with paclitaxel alone or in combination with cannabinoids. These findings suggest that the doses and ratios of THC and CBD used do not induce hepatocellular toxicity.

The route of administration is a critical pharmacokinetic factor that must be carefully considered in animal models. Preclinical research on cannabinoids has traditionally relied on injection methods, such as intraperitoneal, subcutaneous, and intravenous administration. However, oral formulations of cannabinoids, such as THC and CBD, have demonstrated greater safety and are now widely used in medical cannabis treatments (e.g., Dronabinol/Marinol, Epidiolex) (dos Santos et al. 2021). Consistent with this, our study utilized oral administration of cannabinoid formulations at doses of THC and CBD which are equivalent to the doses commonly prescribed to patients for their therapeutic efficacy and tolerability (Gurgenci et al. 2024; Hardy et al. 2024; Inglet et al. 2020; Johnson et al. 2010). In this context, formulations with a balanced THC:CBD ratio (e.g., 1:1) have demonstrated robust analgesic effects with manageable side effects (Hardy et al. 2024; Johnson et al. 2010; Langford et al. 2013; Rog et al. 2007; Serpell et al. 2014).

As the CBD proportion increases (e.g., 1:10 or 1:20), the adverse effects of THC seem to be further mitigated, while maintaining analgesic and anti-inflammatory benefits (Freeman et al. 2019; Pennypacker and Romero-Sandoval 2020).

Although mechanistic studies are beyond the scope of the present work, it could be hypothesized that the mechanisms contributing to the analgesic effects here observed probably involved the activation of CB1 and CB2 receptors, as well as cannabinoid-related receptors such as the serotonin receptor 5HT1A, which modulate nociceptive pathways (Campos et al. 2021). Additionally, both THC and CDB have been proposed to interact with transient receptor potential (TRP) channels, which play a key role in thermal and mechanical pain transmission (Jurga et al. 2024; Muller et al. 2019).

To sum up, this study highlights the differential efficacy of the THC:CBD formulations used in preventing paclitaxel-induced mechanical and thermal pain-related behaviors, as well as the reduction in spontaneous rearing behavior. Additionally, the cannabinoid formulations neither provided therapeutic effects nor exacerbated adverse effects of paclitaxel, such as hypo-locomotion, decreased vertical exploratory activity, heightened anxiety, and attenuated weight gain related to reduced food intake. Moreover, no hepatotoxic effects were observed with the administration of paclitaxel and/or cannabinoids. Therefore, early administration of THC and CBD formulations effectively prevented pain-related behaviors without exacerbating paclitaxel-induced alterations in a chemotherapy-induced neuropathy model. These formulations represent a promising therapeutic strategy to prevent this frequent and severe side effect of chemotherapy treatment, potentially improving the quality of life for cancer patients.

Conclusions

By exploring the effects of two cannabinoid formulations (THC:CBD 1:1 and THC:CBD 1:20) in a rat model, the research identifies promising strategies to prevent and reduce pain-related behaviors. The findings suggest that these cannabinoid combinations can offer targeted relief without additional toxicity, highlighting their potential as therapeutic options. Given that the administration route and doses used were comparable to those in approved cannabinoid-based medications, this study opens news avenues for developing interventions to manage chemotherapy-induced neuropathy, ultimately improving the quality of life for cancer patients undergoing treatment.

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Author contributions DS: Investigation, Methodology, Formal analysis, Conceptualization, Visualization, Writing - original draft, Writing - review & editing. PRB: Resources, Writing - review & editing. MJV: Resources, Writing - review & editing. MFC: Project administration, Conceptualization, Visualization, Supervision, Formal analysis, Writing – original draft, Writing - review & editing, Funding acquisition.

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Data availability The data that support the findings of this study are available from the corresponding author upon request.

Declarations

Ethical approval All procedures were approved by the local Animal Care and Use Committee from Instituto de Investigaciones en Medicina Traslacional (IIMT) CONICET-Universidad Austral, Buenos Aires, Argentina (Assurance number 03/2020 and 04/2020) and were carried out in accordance with all relevant guidelines and regulations.

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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