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Broad-spectrum cannabis oil ameliorates reserpine-induced fibromyalgia model in mice

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ABSTRACT

Fibromyalgia (FM) is an idiopathic disorder characterized by generalized pain and associated symptoms such as depression and anxiety. *Cannabis sativa* shows different pharmacological activities, such as analgesic, anti-inflammatory, neuroprotective, and immunomodulatory. Associated with this, the use of an oil with low concentrations of THC can reduce the psychomimetic adverse effects of the plant. Therefore, the present study aimed to evaluate the analgesic effect of broad-spectrum cannabis oil with low THC concentration in an experimental model of FM. Mechanical hyperalgesia, thermal allodynia, depressive- and anxious-related behavior, and locomotor activity were evaluated after reserpine (0.25 mg/kg; injected subcutaneously (s.c.) once daily for three consecutive days) administration. Our results showed that oral administration of broad-spectrum cannabis oil (0.1, 1, and 3 mg/kg, p.o.) in a single dose on the 4th day inhibited mechanical hyperalgesia and thermal allodynia induced by reserpine. Relevantly, treatment during four days with broad-spectrum cannabis oil (0.1 mg/kg, p.o.) reduced mechanical hyperalgesia 1 h after reserpine administration. Intraplantar treatment with cannabis oil significantly reversed mechanical and heat thermal nociception induced by reserpine injection. Interestingly, spinal and supraspinal administration of broad-spectrum cannabis oil completely inhibited mechanical hyperalgesia and thermal sensitivity induced by reserpine. The repeated cannabis oil administration, given daily for 14 days, markedly mitigated the mechanical and thermal sensitivity during the FM model, and its reduced depressive-like behavior induced by reserpine. In summary, broad-spectrum cannabis oil is an effective alternative to reverse the reserpine-induced fibromyalgia model.

Abbreviations: FM, Fibromyalgia; CNS, central nervous system; THC, tetrahydrocannabidiol; CBD, cannabidiol; MCT, Medium-chain triglycerides; ABRACE, Associaç ão Brasileira de Apoio Cannabis Esperança; CEUA, Animal Ethics Committee; UFSC, Universidade Federal de Santa Catarina; p.o., oral route; i.pl., intraplantar; i.t., intrathecal; i.c.v., intracerebroventricular; ANOVA, analysis of variance; CONCEA, Brazilian Council of Animal Experimentation; g, grams; h, hours; TST, tail suspension test; kg, kilograms; mg, milligrams; min, minutes; ml, milliliters; PBS, phosphate-buffered solution; SEM, standard error of mean; ST, splash test; mmol, millimolar; AUC, area under the curve; µl, microliters; MCT, medium-chain triglyceride oil; s.c., injected subcutaneously; PPAR-α, peroxisome proliferator-activated receptor alpha; TRPV1, transient receptor potential cation channel subfamily V member 1; CB1, cannabinoid receptor 1; FAAH, fatty acid amide hydrolase.

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1. Introduction

Fibromyalgia (FM), a syndrome of unknown etiology, is a complex and chronic disease characterized by generalized muscle pain, accompanied by various somatic and psychological symptoms, e.g., fatigue, stiffness, sleep disturbances, cognitive disorders, irritable bladder syndrome, headache, anhedonia, and depression [1]. Its prevalence is around 0.5–5%, mainly affecting women [2]. It is noteworthy that the biological underpinnings of this condition remain unclear, making it challenging to investigate potential factors that could modulate FM-related symptoms [3]. Although several hypotheses describe this pathology, there is no strong consensus on its pathophysiology, with many aspects still inexplicable [4]. However, it is well-known that FM is strongly related to a central sensitization phenomenon characterized by neurocircuitry dysfunction, which correlates with the perception, transmission, and processing of afferent nociceptive stimuli, thus affecting prevalent manifestations of pain in the locomotor system [5].

The current pharmacological therapy, e.g., neuropathic, anticonvulsant, and antidepressant drugs, predominantly focus on symptomatology [6], with limited efficacy since they cannot contemplate all the repercussions presented by FM patients. Thus, about 90% of patients need to resort to alternative therapies to control symptoms [7]. In this regard, preclinical studies are of great relevance to bringing light to the pathophysiology of FM, and developing new therapeutic targets aiming at promoting novel treatment options, considering the efficacy limitations of the current therapeutic alternatives [7].

In this regard, recent evidence brought to light the use of cannabinoids as an alternative treatment for FM [8,9] . Although the evidence supporting cannabis use in chronic pain conditions is abundant and well established, clinical applicability and safety have not been properly reported in FM [10,11]. For instance, cannabis use is associated with some significant risks, e.g., addiction and psychiatric, cognitive, and developmental impairments [11]. It is well-known that cannabis interacts with the central nervous system (CNS) through endocannabinoid receptors and signaling molecules that produce analgesia and psychomimetic effects. One of the well-known psychoactive substances in cannabis is the tetrahydrocannabidiol (THC) or Δ9-THC [11], which acts as an agonist of endocannabinoid receptors and reduces neurotransmission. However, cannabis also features other naturally occurring phytocannabinoids, e.g., cannabidiol (CBD), that lacks the psychomimetic properties induced by THC [12]. With this in mind, biotechnological developments and extraction techniques have allowed the production of cannabis extracts and oils almost devoid of psychomimetic influence due to the isolation of the psychoactive substances [11]. Therefore, the broad-spectrum cannabis oil is nearly Δ9-THC free, but it contains all of the phytochemicals found in the plant, including terpenes, flavonoids, and other phytocannabinoids such as CBD [13]. Against this backdrop, the present study determined the broad-spectrum cannabis oil effects on reserpine-induced FM in mice, evaluating its analgesic effects using different routes of administration.

2. Materials and methods

2.1. Drugs and reagents

Reserpine was acquired from Sigma Aldrich Chemical Company (St. Louis, MO, USA); Lyrica® (pregabalin) was purchased from Pfizer (New York, NY, USA). Reserpine was diluted in 0.5 % tween 80 (v/v in phosphate-buffered solution; PBS), and pregabalin in saline solution (0.9 % NaCl). Broad-spectrum cannabis oil was diluted with medium-chain triglyceride oil (MCT).

2.2. Broad-spectrum cannabis oil

The broad-spectrum cannabis oil was produced and analyzed by the Brazilian Association ABRACE (Associação Brasileira de Apoio Cannabis Esperança, Paraíba – Brazil/ National Register of Legal Entities - CNPJ under the number 23.877.015/0001–38). The chromatographic analysis was performed and published in a previous study and reported a CBD: Δ9-THC proportion of 11:1 and total cannabinoids of 40.2 % (15 mg of CBD in 1 ml of the oil) [13]. Regarding the microbiological assessment, the oil was under the current quality parameters [13].

2.3. Animals

The experiments were carried out in female Swiss mice (30–40 g, 60–120 days of age, $n_{total} = 120$ animals) since the disease is more prevalent in women, obtained from the Federal University of Santa Catarina. Animals (maximum of 8 mice group-housed in clear transparent plastic cages with dust-free sawdust bedding) were kept under a 12 h light/dark cycle (artificial light on at 7:00 a.m.) and temperature (22 ± 2 °C). They were fed a pelleted and extruded mouse diet ad libitum and had unrestricted access to drinking water. Animals were acclimatized to laboratory settings for at least 1 h before testing and were used only once throughout the experiments. The daily experimental procedures were always performed at the same time and respected the light/dark cycle. All procedures in this study were performed following the ARRIVE 2020 and "Principles of Laboratory Animal Care" from NIH Publication No. 85–23 and were approved by the Animal Ethics Committee of the Universidade Federal de Santa Catarina (CEUA-UFSC, protocol number 2572210218). Behavioral evaluations were performed between 8:00 a.m. and 5:00 p.m.

2.4. Biogenic amine depletion model

The induction of FM was performed using the model proposed by Nagakura and collaborators of depletion of biogenic amines in mice. For this, 0.25 mg/kg of reserpine was dissolved in 0.5 % tween 80 (distilled water and 0.5 % tween 80). Reserpine was injected subcutaneously (s.c.) once daily for three consecutive days in a volume of 10 ml/kg as reported by Nagakura et al. (2009) and later adapted to mice [14,15].

2.5. Experimental design

Firstly, to evaluate the effects of oral administration (p.o.) with broad-spectrum cannabis oil in reserpine-injected mice, treatment with the oil was performed a single administration comparing three different doses of 0.1, 1, and 3 mg/kg, at the end of the induction. In addition, a positive control was performed with pregabalin 30 mg/kg, p.o., a single administration. Mechanical hyperalgesia and cold thermal allodynia were evaluated before induction (baseline), before treatment (0 h), and after 30 min, 1, 2, 3, and 4 h after treatment administration the day following the end of the induction (day four). Thermal allodynia to heat was also evaluated at baseline, 0 h, and after two hours of treatment (Fig. 1 – A). Secondly, we performed the oral administration of broad-spectrum cannabis oil at for four consecutive days (1, 2 and 3rd day of induction and on the 4th day) dose of 0.1 mg/kg on the fourth day after the first reserpine administration, and after that, accessed the baseline mechanical hyperalgesia, 0 h, and after 30 min, 1, 2, 3, and 4 h of treatment. Moreover, thermal allodynia to cold and heat was also accessed at baseline, 0 h, and after two hours of treatment (Fig. 1 – B).

Afterward, three independent experiments were carried out to evaluate the administration of broad-spectrum cannabis oil (or vehicle) through intraplantar (i.pl.) - 100 μg/i.pl. (Fig. 1 – C), intrathecal (i.t.) - 1 μg/i.t. (Fig. 1 – D), or intracerebroventricular (i.c.v) - 2 μg/ i.c.v. (Fig. 1 – E) route. The single dose of broad-spectrum cannabis oil was administered on the fourth day after the first reserpine administration, and after that, mechanical hyperalgesia was accessed at baseline, 0 h, and after 30 min, 1, 2, 3, and 4 h of treatment. Moreover, thermal allodynia to cold and heat was accessed at baseline, 0 h, and after two treatment hours. Finally, a protocol was carried out to evaluate the effects of the chronic administration of broad-spectrum cannabis oil

(1 mg/kg, p.o., daily). The untreated-control group received vehicle (p.o., daily), or positive control group received pregabalin 30 mg/kg, p.o., every day. Treatment started on the fourth day after the first reserpine administration and continued for ten consecutive days. Behavioral test assessments were performed in all groups before day 0 to obtain baseline tactile and thermal thresholds. Subsequently, on days 0, 3 (before treatment), 4, 5, 6, 9, 11, 13, and 14 post-reserpine induction, the animals were submitted to the following behavioral tests: (i) mechanical hypersensitivity – days 0, 3, 4, 9, and 14; (ii) thermal stimulation to cold – days 0, 3, 4, 9 and 14; (iii) thermal stimulation to heat – days 0, 3, 4, 9 and 14; (iv) passive stress-coping behaviors in the tail suspension test – days 6 and 13; (v) anxiety-like behavior in the elevated plus maze test– days 5 and 12;(vi) anhedonic-like behavior in the splash test– days 5 and 12; and (vii) exploratory behavior in the open field test behavior– days 4 and 14 (Fig. 1 – E).

2.6. Behavioral tests

2.6.1. Mechanical hyperalgesia

For evaluation of mechanical hyperalgesia, we used the von Frey test (0.4 g filament – the ventral surface of the right hind paw) [14,15] at different time points (0, 3, 4, 9, and 14 days post-induction).

2.6.2. Acetone test

The acetone test assessed cold allodynia. Using a flat-top syringe, we applied 20 µl of acetone to the plantar surface of each mouse's hind paw through the metallic mesh floor and observed their responses. Responses were monitored for 20 s after acetone application and scored on a four-point scale, as described previously [16]. For example, 0 = no response; 1 = quick withdrawal, flick, or stamp of the paw; 2 = prolonged withdrawal or repeated flicking of the paw; 3 = repeated flicking of the paw with persistent licking directed at the ventral side of the paw. The cumulative scores were then obtained by calculating each mouse's three scores and dividing by five, which was the number of assays [17].

2.6.3. Tail flick

The test consists of a briefly immersion of the tail in hot water (48 ± 1 °C) to measure the thermal threshold's latency [5]. For the animal that did not show nociceptive behavior after 15 s, the stimulus was suspended to avoid tissue damage. Baseline latency (10 s) was determined before testing, and withdrawal latencies were measured manually.

2.6.4. Splash test

This test is based on the evaluation of self-cleaning behavior. Briefly, sucrose solution (200 µl of a 10 %) was squirted on each mouse's dorsal coat inducing grooming behavior. The time and numbers of grooming were recorded for 5 min as the ratio of self-care and motivational behaviors [18,19].

2.6.5. Tail suspension test

Passive stress-coping behavior was assessed using the tail suspension test (TST). Each mouse was suspended 50 cm above the floor, and a small piece of the adhesive tape to a wooden stick near the end of the mice tail about 2 cm, and a barrier surrounded the mice's views. The mice's tail test lasted 6 min. Immobility latency, total immobile time, and the number of immobility were evaluated. Mice were considered immobile when they showed hopelessness, which the mice stopped struggling to overcome the abnormal position, and were nearly immobile or completely motionless after a period of struggling activity [20].

2.6.6. Elevated plus maze test

The elevated plus maze (EPM) was executed according to the protocol previously described [13]. The apparatus consisted of two open arms (35 cm × 5 cm), and two closed arms (35 cm × 5 cm × 15 cm) that extended from a central platform (6 cm × 6 cm). The entire maze was elevated to a height of 50 cm above the floor. Mice were individually allocated in an open arm facing the center of the maze, and the number of entries and time spent in both arms were recorded for 5 min. The increase in the percentage of entries and reduction time spent in the

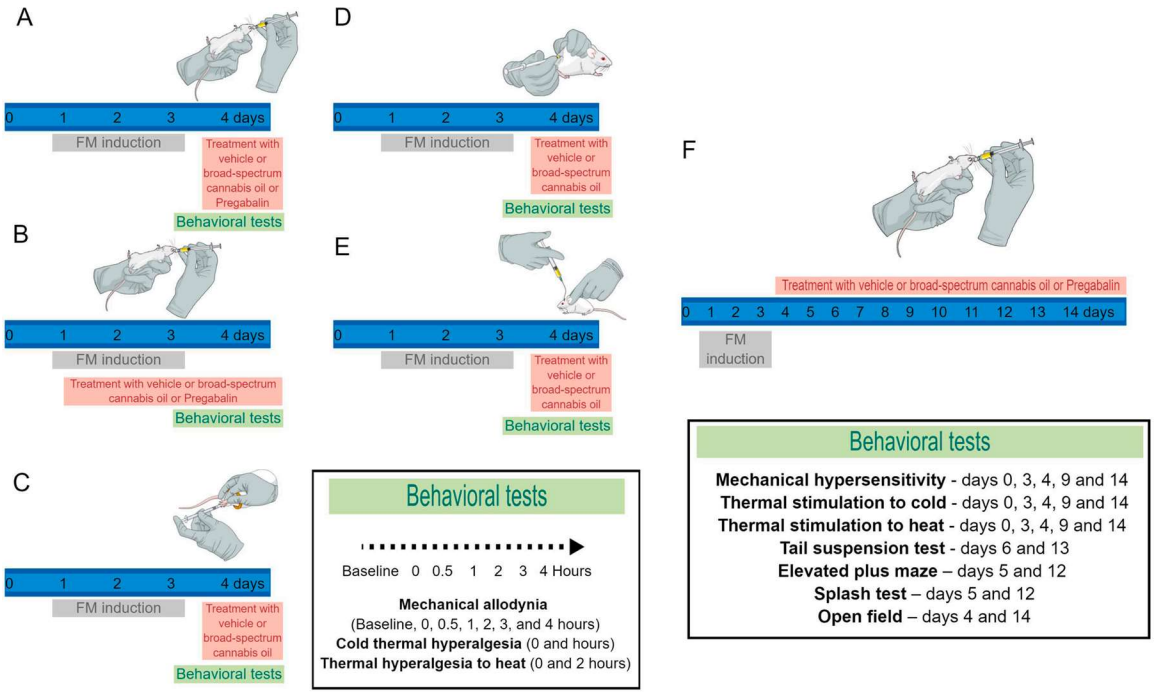

Fig. 1. Experimental design - (A) Broad-spectrum cannabis oil treatment was performed for four days at doses of 0.1, 1 and 3 mg/kg, once a day, p.o. for 4 days. (B) Broad-spectrum cannabis oil treatment was performed at doses of 0.1 mg/kg, once a day, p.o. (C) Broad-spectrum cannabis oil treatment was performed, once a day, 100 µg/i.pl. (D) Broad-spectrum cannabis oil treatment was performed, once a day, 1 µg/i.t. (E) Broad-spectrum cannabis oil treatment was performed, once a day, 2 µg/i.c.v. (F) Chronic treatment with broad-spectrum cannabis oil for 10 days, 1 mg/kg daily p.o.

open arms was considered anxiolytic-like profiles. The apparatus was cleaned out after each animal was evaluated with 10% ethanol solution [13].

2.6.7. Open field test

To investigate the possibility of broad-spectrum cannabis oil treatment developing nonspecific muscle-relaxing and sedative effects during the FM model, we used open-field apparatus (40 cm × 60 cm × 50 cm). The time spent in ambulation and rearing behavior were counted manually for 5 min [18]. The maze was wiped clean after each animal was evaluated with 10% ethanol solution [14].

2.7. Statistical analysis

Results are expressed as the mean ± standard error of the mean (SEM). Results were analyzed by a mixed-model one-way or two-way ANOVA. Normality and homoscedasticity were evaluated using Shapiro-Wilk's and Levene's tests, respectively. One-way (inhibition rate and behavioral test) and two-way (pain assessments) analysis of variance (ANOVA) followed by Bonferroni's post-hoc were performed to analyze the differences among experimental groups and compared with the control group. P values of $< 0.05 < 0.01$ and < 0.001 were considered statistically significant. Data were analyzed using GraphPad Prism software version 9.4.0 for Windows (San Diego, CA, USA).

3. Results

3.1. Effects of oral dose administration of broad-spectrum cannabis oil on reserpine-evoked nociception

First, to assess the therapeutic effect of oral administration of broad-spectrum cannabis oil on the nociception of reserpine-injected animals, the tactile threshold was measured using the von Frey test. In this study, the administration of reserpine (0.25 mg/kg, s.c.) induced a pronounced mechanical hypersensitivity after four days. In this sense, oral administration of broad-spectrum cannabis oil (0.1, 1 and 3 mg/kg, p.o.) in a single dose on the 4th day inhibited mechanical hyperalgesia after two hours of treatment (two-way ANOVA [$F(4, 25) = 8.87$, $p < 0.01$]) (Fig. 2 – **A**), with inhibitions (area under the curve) of 45 %, 42 % and 39 % for doses 0.1, 1 and 3 mg/kg, respectively (one-way ANOVA of treatment effect [$F(4, 85) = 4.47$, $^{***}p < 0.001$] (Fig. 2 – B). Moreover, treatment during four days with broad-spectrum cannabis oil (0.1 mg/kg, p.o.) reduced reserpine-induced mechanical hyperalgesia 1 h after reserpine administration (two-way ANOVA [$F(12, 147) = 2.55$, $p < 0.05$]) (Fig. 2 – F) – inhibition of 23% (one-way ANOVA of treatment effect [$F(2, 12) = 147{,}7$, $^{***}p < 0.001$]) (Fig. 2 – G). Interestingly, a single administration of broad-spectrum cannabis oil (0.1 mg/kg, p.o.) reversed the nociceptive effects caused by reserpine on cold thermal allodynia after two hours of treatment (two-way ANOVA [$F(4, 25) = 5.39$, $^{***}p < 0.01$]) (Fig. 2 – C and D). However, no significant effects on heat thermal allodynia with broad-spectrum cannabis oil were observed (Fig. 2 – E and J). In addition, the four-day oral treatment with the oil did not demonstrate significant effects on cold allodynia (Fig. 2 – H and I).

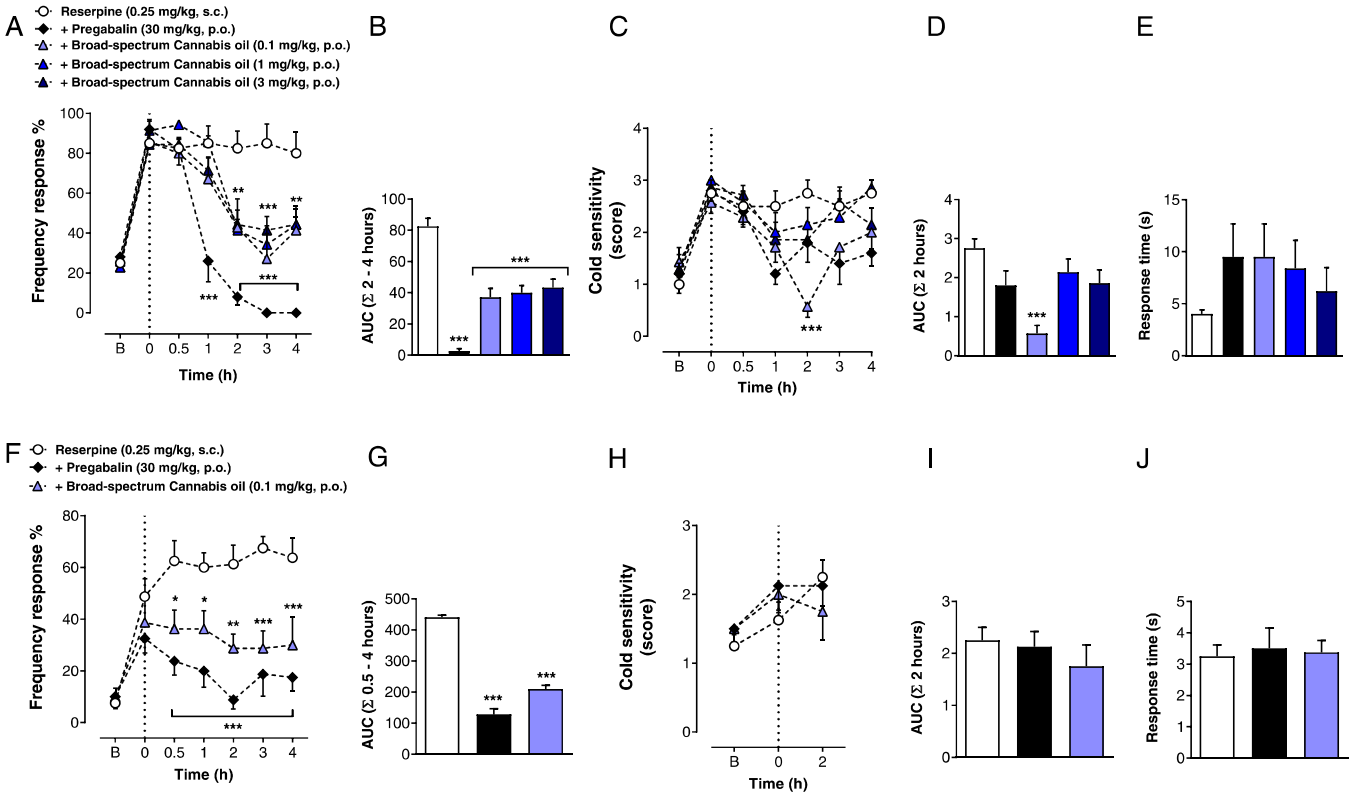

Fig. 2. Anti-hyperalgesic effects of oral broad-spectrum cannabis oil during reserpine model - Oral treatment with broad-spectrum cannabis oil at doses of 0.1, 1 and 3 mg/kg for four days. (A) Von Frey test, (B) area under the von Frey test curve, (C) Acetone test, (D) Area under the acetone test curve and (E) Tail flick test. Single-dose treatment on day four orally with broad-spectrum cannabis oil at a dose of 0.1 mg/kg (F) Von Frey test, (G) Area under the von Frey test curve, (H) Acetone test, (I) Area under the acetone test curve, and (J) Tail flick test. Each line/column represents the mean ± SEM of five and eight mice/group and are representative of two independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. reserpine group (one-way and two-way ANOVA analysis of variance followed by Bonferroni's post hoc test). AUC: area under the curve.

3.2. Effects of intraplantar treatment with broad-spectrum cannabis oil on nociception induced by reserpine administration

Reserpine administration induced a significant decrease in mechanical and thermal nociceptive threshold compared to the untreated-control group. Therefore, in order to investigate the effects of intraplantar administration of broad-spectrum cannabis oil (100 μg) on the nociceptive effects of reserpine-injected animals, treatment was performed on the fourth day (Fig. 3). Intraplantar treatment with broad-spectrum cannabis oil significantly reversed mechanical nociception induced by reserpine injection after 1 and 2 h of treatment (two-way ANOVA; [F (5, 84) = 37.15, $p < 0.01$], Fig. 3 – A), with inhibition of 61% (one-way ANOVA of treatment effect; [F (2, 2) = 1.00, $p < 0.001$], Fig. 3 – B). It was also possible to observe that the oil via intraplantar provided reversal of the nociceptive effects of reserpine on thermal allodynia (two-way ANOVA; [F (2, 42) = 5.91, $p < 0.05$], Fig. 3 – D), although the same effect cannot be observed in the cold thermal nociceptive threshold (Fig. 3 – C).

3.3. Effects of intrathecal and intracerebroventricular administration of broad-spectrum cannabis oil during the FM model

Posteriorly, to evaluate the effects of direct administration of the oil into CNS, two administration routes were evaluated in this reserpine model, intrathecal and intracerebroventricular administration, in doses of 1 μg and 2 μg, respectively. It was possible to assess that i.t. reversed the mechanical nociceptive effects caused by reserpine after 30 min of broad-spectrum cannabis oil administration (two-way ANOVA, [F (1, 10) = 9.96, $p < 0.001$], Fig. 4 – A). This fact can be confirmed with the analysis of the area under the curve that showed inhibition of hyperalgesia by 27% (one-way ANOVA of treatment effect [F (3, 3) = 15.40, $p < 0.01$], Fig. 4 – B). Another important aspect was that the oil via i.t. also showed modulating effects on heat-thermal nociceptive pathways (two-way ANOVA, [F (2, 20) = 53.89, $p < 0.05$], Fig. 4 – D), but not on cold-thermal pathways (Fig. 4 – C). Interestingly, i.c.v. administration only had analgesic effects after 3 h of treatment with oil (two-way ANOVA; [F (6, 72) = 28.96, $p < 0.001$], Fig. 4 – E), showing an inhibition rate of 43% (one-way ANOVA of treatment effect, [F (2, 69) = 0.68, $p < 0.001$], Fig. 4 – F), in addition, no effects of this administration on thermal nociceptive pathways were observed (Fig. 4 – G and H).

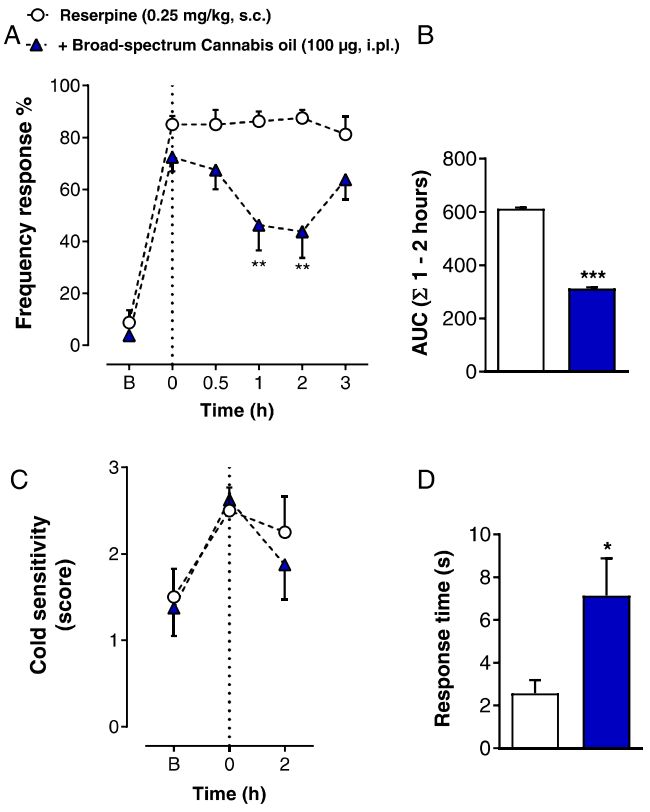

Fig. 3. Anti-hyperalgesic effects of intraplantar broad-spectrum cannabis oil during reserpine model - Single-dose treatment on day four broad-spectrum cannabis oil at a dose of 100 μg, (A) Von Frey test, (B) area under the von Frey test curve, (C) Acetone test, and (D) Tail flick test. Each line/column represents the mean ± SEM of eight mice/group and are representative of two independent experiments. * $p < 0.05$, * * $p < 0.01$ and * ** $p < 0.001$ vs. reserpine group (one-way and two-way ANOVA of analysis variance followed by Bonferroni's post hoc test). AUC: area under the curve.

3.4. Effect of chronic treatment with broad-spectrum cannabis oil on nociception and behavioral disturbances after reserpine administration

Finally, we evaluated the effects of oral administration of broad-spectrum cannabis oil for ten consecutive days and its action on possible nociceptive changes and behavioral disorders related to the induction of fibromyalgia-like by reserpine. The von Frey test demonstrated that after one day of treatment the oil promoted mechanical analgesia (two-way ANOVA; [F (2, 21) = 72.03, $p < 0.001$], Fig. 5 – A), relating an inhibition rate of 45% (one-way ANOVA of treatment effect, [F (2, 69) = 0.68, $p < 0.001$], Fig. 5 – B), however, it did not demonstrate significant long-term effects on thermal nociceptive changes (Fig. 5 – C and D). Interestingly, the broad-spectrum treatment with cannabis oil demonstrated significant effects on anhedonic-like behavior on the second day of treatment, increasing the time and number of groomings (one-way ANOVA of treatment effect [F (5, 42) = 0.67 and F (5, 42) = 0.19 respectively, $p < 0.05$], Fig. 5 – E and F), in addition to decreasing the depressive-like behavior by reducing the immobility time in the TSC (one-way ANOVA of treatment effect [F (5, 42) = 6.90, $p < 0.05$], Fig. 5 – G). However, it was not possible to observe the effects of the oil in the other evaluations (Fig. 5 – H). Also, it was not possible to observe significant effects of oil administration on anxious-like behavior (Fig. 5 – I and J and K). Furthermore, it was also possible to clarify that the administration of oil did not interfere with locomotor activities (Fig. 5 – L).

4. Discussion

Pharmacological options have demonstrated effectiveness in the management of FM, such as amitriptyline (tricyclic antidepressant), duloxetine, milnacipran (serotonin and noradrenaline reuptake inhibitors), tramadol (opioid), gabapentin and pregabalin (anticonvulsant), although most of these drugs provide only modest benefits. Moreover, they are often associated with side effects, compromising adherence [21]. Considering this evidence, the present study evaluated the effects of broad-spectrum cannabis oil on reserpine-induced fibromyalgia-like model in mice. One of the remarkable results obtained herein is that the broad-spectrum cannabis oil, regardless of the route of administration, was effective in mitigating reserpine-induced mechanical and thermal hyperalgesia in mice. Moreover, chronic administration of oil reversed reserpine-induced mechanical and thermal (heat) nociceptive behavior and attenuated reserpine-induced passive stress-coping behavior and lower-self-care behavior in mice.

Firstly, we demonstrated that reserpine injections induced mechanical and thermal nociceptive and depressive-like behaviors in female mice. In this regard, reserpine mimics a complex condition found in FM patients, which involves a dysfunction and depletion of biogenic amines in the CNS [5,14]. It is noteworthy that multiple genetic polymorphisms affect the transmission and processing of pain through serotonergic, catecholaminergic, and dopaminergic mechanisms that may play an important role in the etiology of FM [5,7]. Moreover, the present study observed that reserpine-injected mice showed mechanical hyperalgesia,

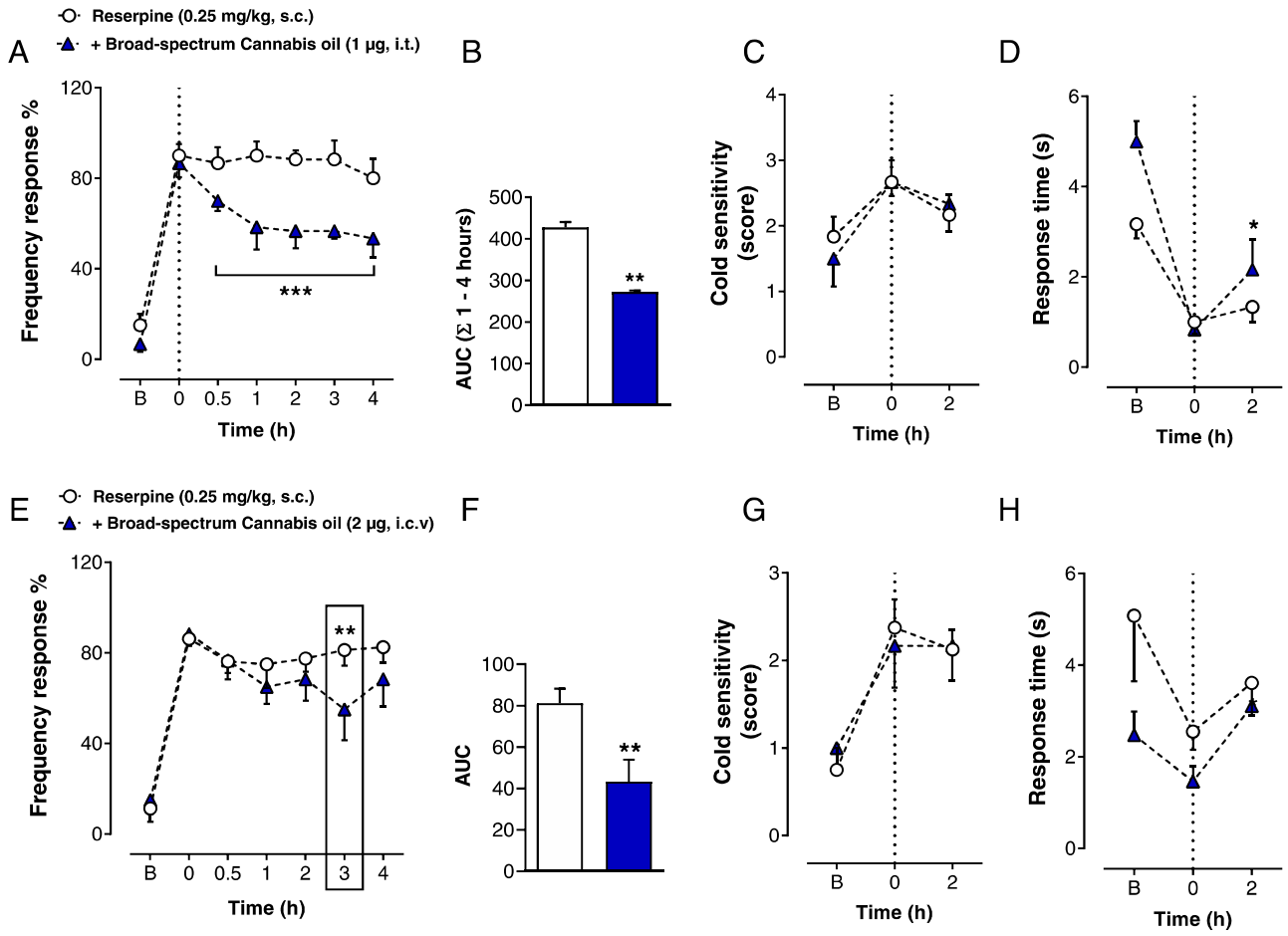

Fig. 4. Anti-hyperalgesic effects of intrathecal and intracerebroventricular administration broad-spectrum cannabis oil during reserpine model - Single-dose treatment on day four broad-spectrum cannabis oil. The effects of intrathecal injection in (A) Von Frey test, (B) area under the von Frey test curve, (C) Acetone test, and (D) Tail flick test. The effects of intracerebroventricular administration in (E) Von Frey test, (F) area under the von Frey test curve, (G) Acetone test, and (H) Tail flick test. Each line/column represents the mean ± SEM of eight mice/group and are representative of two independent experiments. $^{**}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. reserpine group (one-way and two-way ANOVA analysis of variance followed by Bonferroni's post hoc test). AUC: area under the curve.

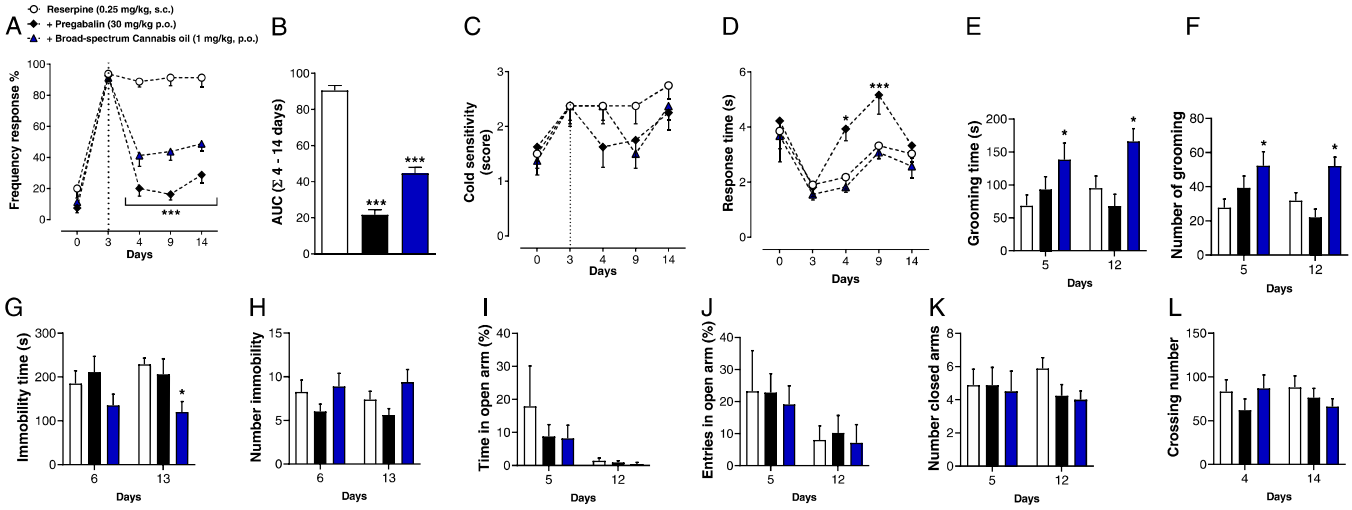

Fig. 5. Anti-hyperalgesic and behavioral effects of oral administration broad-spectrum cannabis oil during reserpine model for 10 days - The effects of administering the oral chronic in (A) Von Frey test, (B) area under the von Frey test curve, (C) Acetone test, (D) Tail flick test, (E and F) Anhedonic-like behavior in time and number grooming, (G and H) Tail suspension test and its effects on the time of immobility and number, (I and J) - Elevated Cross Maze, percentage of time and open arms entries, and numbers of times in closed arms and, (L) Open field test crossing numbers. Each line/column represents the mean ± SEM of eight mice/group and are representative of two independent experiments. $^{**}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ 0.001 vs. reserpine group (one-way and two-way ANOVA analysis of variance followed by Bonferroni's post hoc test and one-way ANOVA). AUC: area under the curve.

a condition predominantly observed in "fibromyalgic" patients. FM patients have a condition called central sensitization and abnormal pain modulation, which seems to be the primary mechanism that causes hypersensitivity to painful stimuli and reduced descending pain inhibition [22]. Herein, we demonstrated that broad-spectrum cannabis oil mitigated the reserpine-induced mechanical hyperalgesia. In agreement with our findings, Gregorio and colleagues [23] demonstrated that administration of CBD intravenously (0.1–1 mg/kg) and subcutaneously (5 mg/kg/day for 7 days) induced mechanical analgesia in neuropathic pain models. Of note, the authors demonstrated evidence that such effects occurred through TRPV1 activation and by potentiating serotonergic neurotransmission. Additionally, FM patients may have potentially impaired pain processing due to changes in connectivity and functional levels of inhibitory and excitatory neurotransmitter concentrations in pain-processing regions of the brain [24]. Thus, FM patients are more sensitive to stimuli, such as heat and cold and mechanical pressure. These stimuli provoke a pain response that would not otherwise be triggered in healthy individuals [11]. In addition to the mechanisms mentioned above, it is believed that there is an association between the hyperalgesia found in patients with FM and the presence of central endocannabinoid hypofunction in the spinal cord [11]. In this sense, recent report has elucidated the involvement of the endocannabinoid system in FM models. For instance, ASP8477, a selective inhibitor of fatty acid amide hydrolase (FAAH, a primary catabolic enzyme for anandamide) restored muscle pressure thresholds during reserpine-induced fibromyalgia model in rats, thus representing a promising pharmacological target for the FM [25]. Thus, one might posit that the broad-spectrum cannabis oil can interact with the endocannabinoid system and thus modulate the nociceptive pathways, resulting in the analgesic effects observed herein. A possible therapeutic target associated with the oil used is the potential of CBD to regulate the levels of anandamide, which in turn acts as an intracellular messenger, amplifying the influx of calcium via TRPV1 channels, thus controlling the release of neurotransmitters, and modulating the nociceptive signal [26].

An important fact in this study is the constitution of the studied oil, which contains few concentrations of THC, therefore preventing possible side effects related to this compound. As previously mentioned, THC, the main psychoactive constituent of cannabis, activates CB1 and CB2 receptors, consequently inducing central and peripheral neuronal activity, and is responsible for the analgesic and psychomimetic effects of cannabis [27]. As much as THC has great clinical effects, a study has shown that, unfortunately, most patients who used THC (2.5–15 mg daily for three months) as therapeutic form gave up early due to THC side effects, but those who completed it had marked reductions in visual analog scales (VAS) of subjective pain [10]. The broad-spectrum cannabis oil studied here has low levels of THC and higher levels of CBD [13], the main non-intoxicating constituent of cannabis [27]. CBD, because it has a low affinity for CB1R, does not represent the psychomimetic effects found by the action of THC. Therefore, it can potentiate the analgesic effects [27]. Moreover, recently our group showed no effects of broad-spectrum cannabis oil in the tetrad behavior – a very useful assay to characterize potential psychomimetic effects of cannabinoids [13], confirming analytical parameters, according to the manufacturer's information (CBD:Δ9-THC proportion of 11:1 and total cannabinoids of 40.2 %).

In the present study, we evaluated different routes of administration, being them oral, intraplantar, intrathecal and intracerebroventricular. Comparing the routes studied here, it can be observed that for mechanical hyperalgesia, oral and intrathecal administrations showed control of hyperalgesia in the first 30 min after treatment. In studies by Kiso et al., similar effects were demonstrated, however, with a single oral administration of ASP8477 (0.3, 1 and 3 mg/kg), a FAAH inhibitor, it demonstrated effectiveness in reversing mechanical hyperalgesia at doses of 1 and 3 mg/kg in the RIM model [25]. This corroborates to establish the oral route as a positive therapeutic target in mechanical sensitivity in this model. In the heat allodynia, routes of administration that showed significant effects were intraplantar and intrathecal, and other routes did not represent antinociceptive effects. Much remains to be clarified about the thermal analgesic effects of cannabinoid therapeutic targets in peripheral and central pathways, mainly related to chronic pain models such as FM. In this sense, other studies using neuropathic pain and inflammatory pain models have investigated possible mechanisms using microinjections of CB1R, TRPV1 and PPARα antagonists before a microinjection of URB597 in the insular cortex, an important brain region involved in pain and emotion processing in the brain. It was possible to observe that blocking CB1R and PPARα, but not TRPV1, reversed antinociceptive effects of URB597 [28,29]. Thus, demonstrating that, possibly, the analgesic effects of the studied oil may be correlated with inflammatory regulation and with the activation of the endocannabinoid system, but hardly with the control of heat sensitivity. Interestingly, only the oral administration of 0.1 mg/kg (single dose) showed repair in cold allodynia after two hours of treatment. Such a result can also be observed with the intraperitoneal administration of a FAAH inhibitor – URB597, whereas the authors correlate the antinociceptive effects with increased levels of endocannabinoids such as anandamide and 2-arachidonoyl glycerol [30]. Moreover, it was observed that the oil administered intrathecally showed effect after half an hour of treatment on mechanical analgesia. This fact may be related to the alterations that reserpine causes at the spinal level since the myalgia model induced by reserpine induces area of interstitial edema in the spinal cord, and a reduction in the area of motoneurons in the ventral horn of the spinal cord [14]. Clinically, cannabinoids are most commonly prescribed for oral or inhaled administration, but these different routes of administration have diverse side effects. These include drowsiness, dizziness, sedation, torpor/disorientation, fatigue/tiredness, nausea, poor coordination, decreased concentration, headache, hypervigilance, edema, insomnia, and increased appetite [31]. However, the main psychomimetic side effects were related to the administration of the product via inhalation due to the effects of THC, and these effects are amplified when consumed through methods with faster absorption, such as smoking or vaping, compared to oral injection [32,33]. Furthermore, pharmacokinetics and pharmacodynamics differ between inhalation and oral ingestion, influencing onset and duration of adverse effects of each method of administration [34]. For this reason, it is important to evaluate different forms of administration to determine which prescription could bring the best result for these patients.

The endocannabinoid system is associated with multiple biochemical actions, modulating not only pain and inflammation, but also emotions, anxiety, and stress. Baseline differences in endocannabinoids and N-acetylethanolamines levels were reported among FM compared with healthy people [26]. Unlike the present study's findings, many authors have demonstrated the anxiolytic effects of CBD administration. CBD has proliferative effects on hippocampal progenitor cells by increasing anandamide levels and is mediated by secondary activation of CB1 and CB2 receptors [35]. Furthermore, since anandamide is known central neuromodulator that is involved in the extinction of traumatic memories, it can be speculated that changes in anandamide levels have repercussions on structural changes in the brain, especially with emphasis on structural changes in the amygdala and hippocampus of the brain [36]. Emphasizing these findings, de Assis et al. [13] demonstrated anxiolytic effects of broad-spectrum cannabis oil (0.1 mg/kg, p.o.) in acute and chronic stress models, stating that the present oil has anxiolytic potential; however, in different experimental models. Another mechanism associated with the antidepressant and anxiolytic effects of CBD is its role in regulating serotoninergic neurotransmitters [37,38].

The fibromyalgia-like model reproduced in the present study proved to be an important tool for assessing anxious and depressive-like behavior. Numerous studies using the RIM model reported increased immobility times in the forced swimming test and tail suspension test and decreased swimming times in the forced swimming test, indicative of depressive-like behavior in rats and mice [39]. An important finding

in this study was that the chronic administration of broad-spectrum cannabis oil had repercussions on analgesic effects and reversed the reserpine-induced passive stress-coping behavior and lower-self-care behavior in mice. Given the data discussed here, it is possible to assume that cannabinoids can alter pain processing, reduce low-grade inflammation, and allow the modulation of emotional and cognitive function in FM patients, as they have anti-inflammatory, antiepileptic, anti-ischemic, and antiemetic properties. However, it is difficult to compare the effects of the oil studied in the present study with other studies already published since medical cannabis, even though it is considered safe and well-tolerated, has a great diversity of cannabis species and different methods of preparation. In this regard, more research is needed to determine the effects of the cannabis drug/dosage used in research and clinical settings [33].

In the present study, it was possible to observe that, regardless of the route of administration, broad-spectrum cannabis oil proved to be effective in reversing the mechanical hyperalgesia effects of the reserpine-induced fibromyalgia model. Furthermore, chronic treatment with broad-spectrum cannabis oil showed analgesic effects on mechanical hyperalgesia and heat allodynia and mitigated reserpine-induced passive stress-coping behavior and lower-self-care behavior in mice. Conjointly, our results point to broad-spectrum cannabis oil as a therapeutic alternative for the disorders caused by FM. Clearly, the findings of the present study may help in the therapy for patients with FM, however, some administration routes studied here are difficult to transpose to clinical practice. Another limiting point of this study is the fact that it does not address possible mechanisms by which broad-spectrum cannabis oil acts. In this sense it is necessary to develop more studies that answer such gaps.

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CRediT authorship contribution statement

Study design and concept, and manuscript writing: EGF, ELGM, RC, RCD; experiment implementation and manuscript drafting: EGF, RSP, GMB, PMA, MCG, PDC, RC, RCD; figure production: EGF, RCD; data analysis: EGF, RSP, GMB, PMA, MCG, NRBR, RC, ELGM, RCD; experiment support: EGF, RSP, GMB, PMA, MCG, PDC, NRBR, RC, ELGM, RCD. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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