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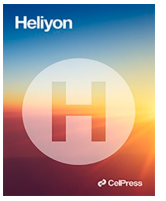

Research article

Behavioral effects of two cannabidiol and cannabigerol-rich formulas on mice

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ABSTRACT

Cannabis sativa L. produces more than 100 specific bioactive compounds, known as cannabinoids. The major non-psychotropic *Cannabis* constituent is cannabidiol (CBD), which displays beneficial properties in a variety of medical conditions. However, the potential therapeutic role of other minor phytocannabinoids, such as cannabigerol (CBG), and their use in combination with CBD, has remained largely unexplored. In this study, we wanted to assess the *in vivo* effects of two novel non-psychotropic cannabinoid formulas, both containing relatively high percentages of CBD but differing mainly for CBG content, hereafter called CBG+ and CBG-formulas. We employed different behavioral tests to evaluate the effects of these formulas at three different dosages on mice locomotor activity, anxiety-related behaviors, short-term memory and sociability. We found that these two formulas display unique behavioral profiles: CBG + formula produced an increase in mice locomotor activity and displayed anxiolytic properties, whereas both formulas improved spatial short-term memory and social interactions. The results obtained suggest that different combinations of phytocannabinoids are able to determine different behavioral effects and highlight the importance of studying the effects of less known phytocannabinoids (like CBG), which used in combination with other phytocannabinoids can change the profile of action of other active compounds (such as CBD).

1. Introduction

Cannabis sativa L. (*C. sativa*) is a herbaceous flowering plant that has been used for millennia for medicinal, therapeutic and recreational purposes. It comprises a variety of chemical constituents, including specific bioactive compounds known as cannabinoids [1]. To date, more than 100 cannabinoids have been identified [2]. The two major phytocannabinoids isolated from *C. sativa* are Δ^9-tetrahydrocannabinol (Δ^9-THC or THC) and cannabidiol (CBD). THC is the predominant psychotropic component of the plant; on the contrary, CBD is a non-psychoactive cannabinoid. Cannabinoids are biosynthesized as cannabinoid acids, and later decarboxylated into their neutral forms. In particular, alkylation of olivetolic acid with geranyl-pyrophosphate by a prenyltransferase produces cannabigerolic acid (CBGA). CBGA generates Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA) and cannabidiolic acid (CBDA) that are precursors of THC and CBD, respectively. CBGA converts also to cannabichromenic acid (CBCA) that is the precursor of another phytocannabinoid called cannabichromene (CBC) [3,4].

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CBD, in particular, is known for its non-psychoactive proprieties and its wide range of potential therapeutic applications. CBD interacts with the endocannabinoid system (ECS), a complex cell-signaling system involved in regulating various functions like mood, pain, immune responses, and homeostasis [5]. CBD has a low affinity for both CB1 and CB2 cannabinoid receptors. Unlike the agonist activity displayed by the major psychoactive cannabinoid, Δ^9-THC, CBD functions as a negative allosteric modulator, reducing the binding efficiency of Δ^9-THC and other agonists to these receptors [6]. Additionally, CBD has broader pharmacological targets beyond CB receptors, such as TRPV1, involved in pain perception, and serotonin receptors, contributing to its anti-inflammatory, analgesic, and anxiolytic properties [7,8]. In recent years, there has been a growing research interest for CBD's potential therapeutic applications for a variety of medical conditions, including complex neurological and psychiatric disorders [9–15], given its anti-inflammatory, antioxidant, anti-tumoral, anxiolytic and antidepressant properties and the lack of psychotropic activity [9]. In particular, research has been focusing on investigating the potential for this particular class of compounds in treating neurodevelopmental disorders, many of which are associated with intellectual disability and significant cognitive and behavioral difficulties. Medical-use CBD (Epidyolex®) has been proposed for the treatment of severe behavioral alterations in patients with tuberous sclerosis complex, mucopolysaccharidosis type III and Fragile X syndrome, with the objective to address the unmet medical needs for effective treatment of these rare genetic conditions using a personalized trial design [16]. A recent systematic review conducted by Parrella and colleagues [17] assessed randomized controlled trials on CBD for treating neurodevelopmental disorders, finding preliminary evidence of potential benefits. These preliminary findings have been corroborated by other reviews, which provide additional evidence supporting the potential efficacy of CBD in neurodevelopmental disorders, though further rigorous studies are still needed to confirm these effects definitively [18,19].

However, most studies have focused on the use of CBD as a stand-alone therapeutic, and research on the potential effects of other lesser-known cannabinoids remains insufficient.

C. sativa is source of several minor phytocannabinoids such as cannabigerol (CBG), cannabinol (CBN), cannabichromene (CBC), Δ9-tetrahydrocannabivarin (THCV), cannabivarin (CBV) and cannabidivarin (CBDV). The spectrum of their pharmacological properties is limited, but recent studies have shed light on their beneficial effects on human health (for a review, see Ref. [15]).

In this work, we wanted to gather evidence on the effect of mixtures of various phytocannabinoids in different proportions. Specifically, we wanted to assess whether the behavioral effect of CBD, the major component of these formulations, is modulated by coupling it with other cannabinoids. Indeed, at the preclinical level the effects of a CBD-based treatment are well-known, as it has been proven to decrease anxiety [20], to improve memory [21], ameliorate autism-like behaviors [22] and to reduce cognitive deficits in rat models of Fragile X syndrome [23]. CBD also displays a prosocial effect both in wild-type mice [24] and mouse models of autism spectrum disorders [25]. Conversely, while there are some studies that have explored the behavioral effects of non-psychotropic Cannabis compound mixtures, the research is still limited and further investigation is needed to fully understand their potential impacts. Pioneering studies in this fields have shown how the combination of CBD and THC, rather than the stand-alone use of either of these two compounds, is effective in the treatment of conditions such as multiple sclerosis [26,27] and seizures [28]. The investigation into the potential use of phytocannabinoid in combination has led to the development of Sativex ®, a herbal formulation containing equal parts of CBD and THC, used in the treatment of patients affected by multiple sclerosis [27].

The formulas tested in the present study contain various phytocannabinoids in different ratios (for the formulas' compositions, see Table 1). In particular, the two formulas contain relatively high percentage of CBD and several other phytocannabinoids in smaller quantities. The two formulas differ mainly in the CBG content: once contains a low percentage of CBG and the other a relatively high percentage. From here on the two formulas will be called CBG- and CBG + formula, respectively. This cannabinoid has recently come to light for its beneficial effects in a variety of conditions, such as neurological, metabolic and gastrointestinal diseases [29,30]. Its carboxylic acid form (CBGA) derives from the combination of geranyl pyrophosphate and olivetolic acid [30]. Decarboxylation of CBGA results in CBG [30]. Growing evidence supports the idea that CBG displays potential therapeutic effects in different pathological conditions [31,32]. For instance, studies have shown that CBG (or its derivatives) exerts anti-inflammatory and neuroprotective properties both *in vitro* [33,34] and *in vivo* [35–39]. CBG operates through a mechanism of action similar to that of CBD, as it binds weakly to CB1 and CB2 receptors [40]. Besides inhibiting CB1R, it also antagonizes the 5-HT1A receptor, activates alpha-2 adrenoceptors, and modulates endocannabinoid signaling [41]. Moreover, CBG can activate TRPV1 and desensitize it, thereby blocking the transmission of pain signals. Additionally, CBG can activate CB2R, but not CB1R, leading to the release of β-endorphin, which significantly enhances its antinociceptive effect [42].

Due to its action on TRPV channels, CBG has been investigated as a potential therapeutic agent in gastrointestinal inflammation and

Table 1
Composition of tested non-psychotropic cannabinoid formulas (CBG- and CBG+ formulas). The solvent is castor oil.

	CBG- formula	CBG+ formula
	%	%
CBD	**45.9**	**37.1**
CBG	**2.5**	**16.0**
CBC	**8.0**	**6.3**
Δ^9-THC	**0.007**	**0**
others phytocannabinoids	**16.3**	**13.9**
solvent	**27.29**	**26.7**

related disorders, such as colitis, with positive outcomes [43,44]. In addition, some research studies have shown that CBG possesses analgesic and pain-relieving effects, mediated by agonistic activity on the alpha-2-adrenoreceptor [45–48]. Moreover, CBG has been shown to ameliorate neurotoxicity derived by cellular oxidative stress [49] and it has been tested as anti-tumoral agent [50]. In addition to this, it has been demonstrated that CBG and CBD, coupled together, display potent neuroprotective and anti-inflammatory properties, primarily mediated through the action on the 5-HT1A receptors [49,51]. Despite emerging evidence for CBG as a potential anti-inflammatory and neuroprotective drug, research on CBG's effects on behavior has been neglected.

Considering the abovementioned data, we wanted to investigate the effects of two novel cannabinoid-based formulas in mice after acute and sub-chronic treatments, using three increasing dosages. The compounds' effects were assessed by a battery of different behavioral tests that allowed us to evaluate whether the formulas were able to induce alterations in distinctive realms of behavioral and cognitive functioning such as locomotor activity, memory, sociability, anxiety and exploratory behavior.

2. Materials and methods

2.1. Animals

All experiments were performed according to European Communities Council Directive guidelines (CEE N° 86/609) and all protocols were approved by the Italian Ministry of Health, Animal care and use Committee of the University of Brescia. Adult male mice (B6; 129PF2) aged 3–6 months (body weight 25–35 gr) were used for our experiments. They were housed 2 to 4 per standard cage (15 cm wide × 35 cm long × 12 cm deep) in a 12 h light/dark cycle (light phase: from 8:00 a.m. to 8:00 p.m.) with food and water available *ad libitum.* Temperature (22 °C) and humidity (50 ± 10 %) in the cage were automatically regulated by the Sealsafe Aero System by individually ventilated cages with EPA filters (Tecniplast Group, Italy). Animals were obtained in our animal facility from mating mice; breeder mice were used only for mating and not for testing. A total of 49 mice were employed for the current study, 7 mice for each of the following experimental groups: vehicle (control group), 25 mg/kg of CBG-formula, 50 mg/kg of CBG- formula, 100 mg/kg of CBG-formula, 25 mg/kg of CBG+ formula, 50 mg/kg of CBG+ formula, 100 mg/kg of CBG+ formula. Mice were first tested after 1 h of acute treatment, and the same mice were tested again after 10 days for sub-chronic treatment tests (see Fig. 1 for a schematic timeline). Treatment and tests were performed during the light phase of the circadian cycle.

2.2. Formulas and treatment

CBG- and CBG+ formulas used for behavioral tests were kindly provided by Ukibori (RENACT) from Cyprus. Formulas composition is described in details in Table 1. For each formula tested, mice were divided into experimental groups (4 groups, 7 mice each). Control group mice were treated with vehicle (~150 μl medium-chain triglyceride oil; Waldo Health, Norderstedt, Germany), and phytocannabinoid formulas treated mice received the following doses: 25 mg/kg, 50 mg/kg or 100 mg/kg of formula. Both vehicle and

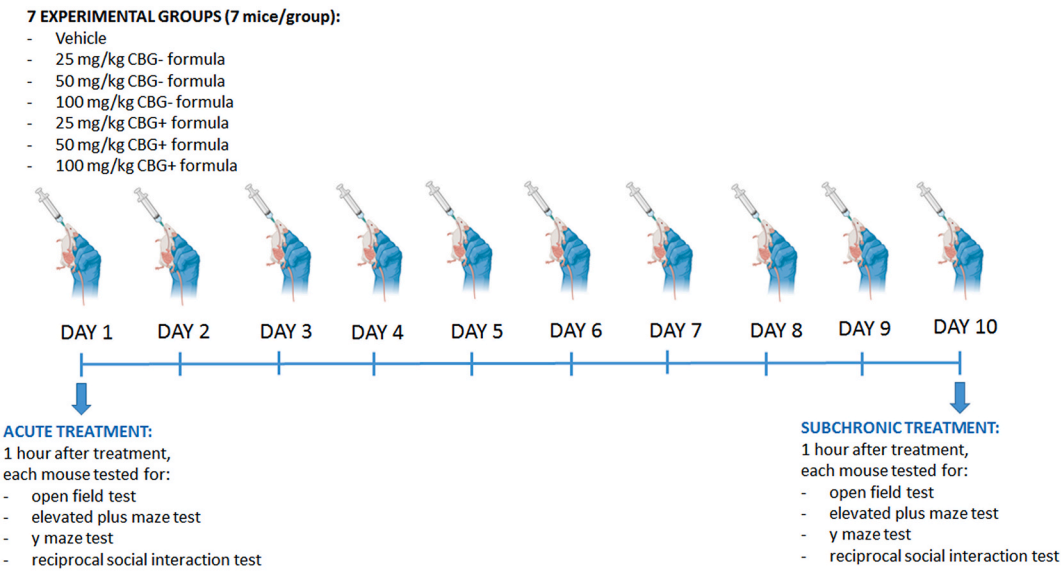

Fig. 1. Schematic timeline of the treatment and behavioral test. B6; 129PF2 mice were divided into 7 groups (control, 25 mg/kg of CBG- formula, 50 mg/kg of CBG- formula, 100 mg/kg of CBG- formula, 25 mg/kg of CBG+ formula, 50 mg/kg of CBG+ formula, 100 mg/kg of CBG+ formula). For all the groups, behavioral testing of the acute administration took place 1 h after the start of the treatment or vehicle. Mice were then tested for the open field, elevated plus maze, Y maze and reciprocal social interaction test. The same mice continued to receive treatment for 10 consecutive days, in which they received the abovementioned doses once a day. The tenth day, 1 h after treatment, they were behaviorally tested for the open field test, the elevated plus maze test, the Y maze test and the reciprocal social interaction test.

formulas were administered via oral gavage. Acute treated mice were treated once and then tested; the treatment was then continued (sub-chronic treatment) for 10 consecutive days, administering the formula once a day. Behavioral tests were performed within 1 h of drug administration, on the acute treatment days and on the last day of the chronic treatment (see Fig. 1).

2.3. Behavioral tests

To assess the behavioral profile resulting from the administration of the two formulas, we utilized a comprehensive battery of diverse behavioral tests. These tests were designed to probe various aspects of mice cognition and motor activity across distinct domains.

Open field. To evaluate locomotor activity, we performed an open field test according to previous protocols [52,53]. In brief, mice were put in the testing room for 10 min before the test to acclimatize to the environment. Afterwards, each mouse was positioned in the center of the arena (40 × 40 cm plexiglass) and they were individually video-recorded for 5 min by a video camera vertically mounted 1.5 m above the arena. EthoVision XT software (version 14.1; Noldus, Wageningen, The Netherlands) was used to automatically track distance travelled and average speed.

Elevated plus maze. To assess anxiety-related behaviors, we employed the elevated plus maze (EPM) test as performed in previous works [24,54]. Mice were placed at the center point of a platform (5 × 5 cm) from which two open arms (35 × 5 cm) and two closed arms (30 × 5 × 15 cm) extend. The apparatus is 50 cm elevated above the floor and each mouse is individually placed in the center point of the EPM, with its nose facing one of the open arms. 5 min videos were recorded via a portable camera placed 1.5 m above the maze. Behaviors of interest were automatically acquired using EthoVision XT software (version 14.1; Noldus, Wageningen, The Netherlands). As an anxiety-related indicator we used open arm exploration, which correlates with low anxiety levels [55]. In particular, we evaluated: percentage of entries in open arms = (number of entries into open arms/number of entries into open and enclosed arms) · 100 %; percentage of time spent in open arms = (time spent in open arms/time spent in open and enclosed arms) · 100 %.

Y maze. The Y maze test was performed to assess cognitive functioning and working memory abilities in mice [53,56]. The apparatus consists of a Y-shaped maze, with three white-colored arms (15 cm height x 8 cm width x 30 cm length) positioned at 120° angles between each other. Mice were placed at the extremity of one arm and allowed to explore the maze freely for 5 min. Each trial was recorded and automatically analyzed by EthoVision XT software (version 14.1; Noldus, Wageningen, The Netherlands). Number of arm entries and alterations were tracked. An entry is defined as the moment the mouse enters an arm with all four limbs; an alteration occurs when the mouse consecutively entries all three arms, without revisiting the first arm. These parameters correlate with working memory functioning since they show whether the mouse is able to recall arms previously visited.

Reciprocal social interaction test (male-female). Male mice are isolated for 48 h prior to the test, according to previous protocols

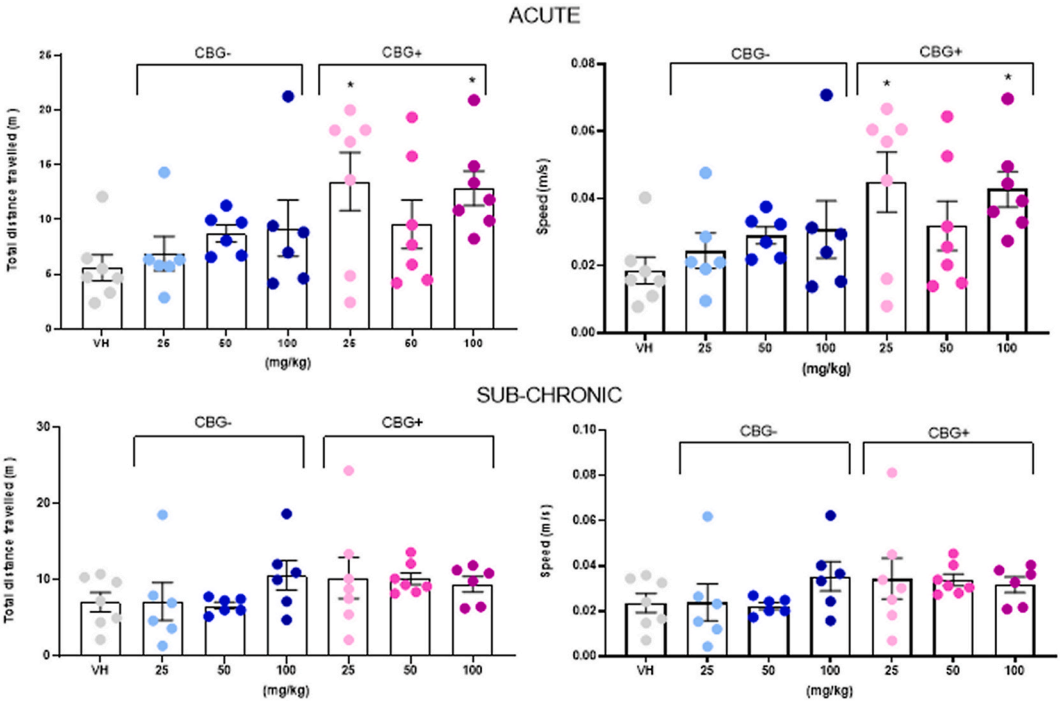

Fig. 2. Effects of CBG- and CBG+ formulas on mice locomotor behavior. Open field test was used to evaluated mice locomotor behavior. The effects of CBG- and CBG+ formulas on mice distance travelled and speed are reported for acute treatment (up) and for sub-chronic treatment (down). Data are expressed as mean ± S.E.M. * = $p < 0.05$ with one-way ANOVA followed by Dunnett's multiple comparison test.

[57]. Individually caged mice were allowed to acclimatize to the testing environment for 10 min. After the environmental habituation, mice were exposed to an unfamiliar female subject, matched for age, sex and strain, for 5 min. Females were tested beforehand to check their vaginal estrous phase and then the females in estrus distributed evenly between the groups. During this time, pair interactions were recorded and subsequently manually analyzed by an operator using the Observer XT software (version 14.1, Noldus, Wageningen, The Netherlands). Behaviors were divided into two major groups: social (anogenital sniffing, body sniffing, nose sniffing, contact, following) and non-social (cage exploring, self-grooming, digging, rearing) and for each individual behavior, time spent in the activity was evaluated.

2.4. Statistical analysis

All the results are presented as the mean ± S.E.M. All statistical analyses were performed using GraphPad Prism 7 software (GraphPad Software, San Diego, CA, USA). The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test for all behavioral tests. Statistical significance was indicated as * $p < 0.05$ and **$p < 0.01$ compared to the control (VH).

3. Results

3.1. Acute treatment with CBG+ formula, but not CBG- formula, produces an increase in mice locomotor activity

In order to evaluate the effects of the two cannabinoid mixtures on motility, mice were treated via oral gavage for one day (acute treatment) or for 10 consecutive days (sub-chronic treatment), once a day, with increasing dosages of either CBG- or CBG+ formulas (25 mg/kg, 50 mg/kg, 100 mg/kg) or vehicle (VH). Performance was evaluated using the open field test. As shown in Fig. 2, CBG- formula treatment did not produce any significant effect in mice locomotor activity, both in acute and in sub-chronic administration. Indeed, no significant differences emerged in both distance travelled and speed (for results, see Supplementary Table 1) (Fig. 2). On the contrary, CBG+ formula induced an overall increase in locomotor activity. After acute treatment, we observed an increase in both total distance travelled and speed, that reached statistical significance for the 25 mg/kg and the 100 mg/kg dosages (for results, see Supplementary Table 2). Lastly, the sub-chronic treatment with CBG+ induced an overall increase in locomotor activity in comparison to vehicle and CBG- formula, although the results did not reach statistical significance either for distance travelled and speed (Fig. 2 and Supplementary Table 2).

The results obtained demonstrate that only the formula with relatively high CBG content affects locomotor behavior increasing

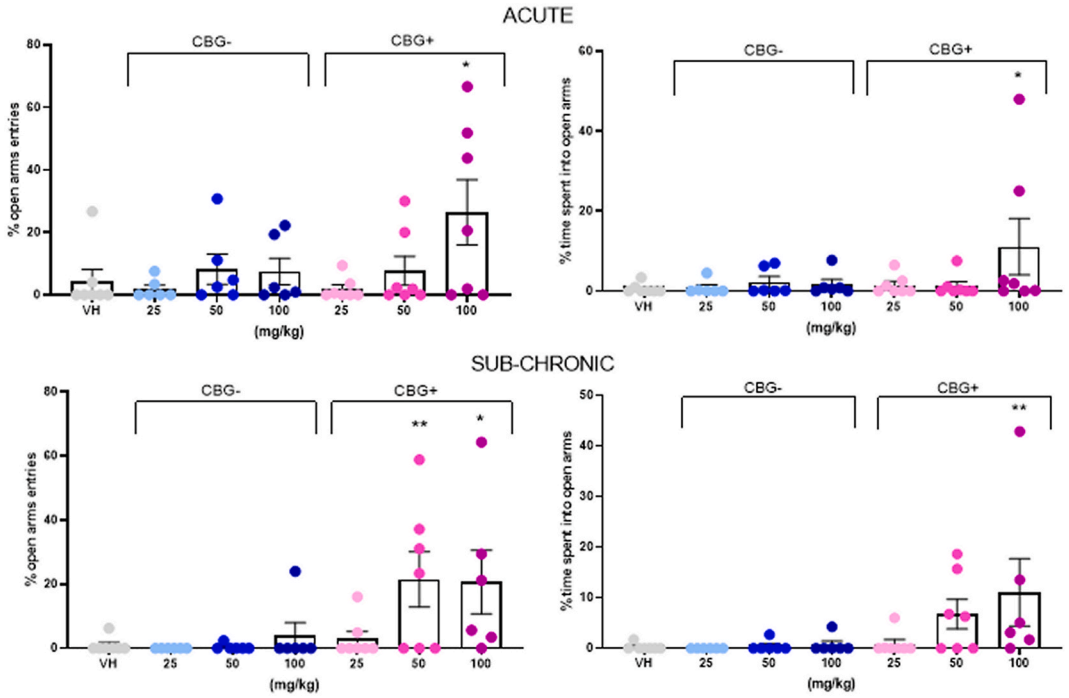

Fig. 3. Effects of CBG- and CBG+ formulas on mice anxiety. Elevated plus maze test was used to evaluated mice anxious behavior. The effects of CBG- and CBG+ formulas on % of open arms entries and % of time spent in open arms are reported for acute treatment (up) and for sub-chronic treatment (down). Data are expressed as mean ± S.E.M. * = $p < 0.05$ and ** = $p < 0.01$ with one-way ANOVA followed by Dunnett's multiple comparison test.

distance travelled and speed, suggesting that CBG affects the modulation of motor activity in mice.

3.2. Both acute and sub-chronic treatment with CBG+ formula, but not CBG- formula, ameliorates anxiety behaviors

We analyzed mice performance on the elevated plus maze, a functional and standardized tool to assess anxious behavior. This test relies on the rodent's natural tendency to prefer dark, enclosed spaces (the closed arm) and the innate avoidant behavior towards heights and open spaces [55], hence a drug displaying anxiolytic properties would lead to an increase in number of entries in open arms and time spent on them.

Regarding the CBG- formula, no significant difference was observed between groups, on both parameters evaluated, either for the acute and the sub-chronic treatment (results are shown in Supplementary Table 3) (Fig. 3). On the other hand, both acute and sub-chronic treatment with the CBG+ formula displayed a dose-dependent anxiolytic effect in mice, that resulted to be significant in the acute-treated 100 mg/kg group and in the sub-chronic treated 50 mg/kg and 100 mg/kg groups (results are shown in Supplementary Table 4) (Fig. 3). The CBG+ formula increased dose-dependently both the open arm entries and the time spent in open arms, thus suggesting a significant contribution of CBG in the anxiolytic effect observed treating mice with the compound with high CBG content.

3.3. Both CBG- and CBG+ formulas, administered acutely, produce dose-dependent improvement in mice spatial memory

We assessed changes in cognitive functioning employing the Y maze, a commonly used test to evaluate spatial working memory in rodents. The performance is based on the mouse's capacity to remember which arm has not been visited previously and its innate disposition to explore unknown areas of the maze, thus the tendency to enter a novel arm each time [56]. Regarding the CBG- formula, a significant increase in arm alternation was observed in the 100 mg/kg treated compared to vehicle-treated mice, and only in the acute treatment (results are shown in Supplementary Table 5) (data are reported in Fig. 4). This increase in spatial memory, was not maintained in CBG-formula chronically treated mice (Supplementary Table 5).

For the CBG+ formula, Fig. 4 displays a significant dose-dependent positive effect on short-term spatial memory, as indicated by the number of arms alternation in the acutely administered 50 mg/kg group and 100 mg/kg groups compared to the vehicle-treated group (data are shown in Supplementary Table 6). This effect was no more present after sub-chronic administrations (Supplementary Table 6). These results show that both CBG- and CBG+ formulas produce a significant improvement in learning and memory, measured as short-term working memory, as indicated by the alternation pattern in exploring the different arms of the maze. This effect was not

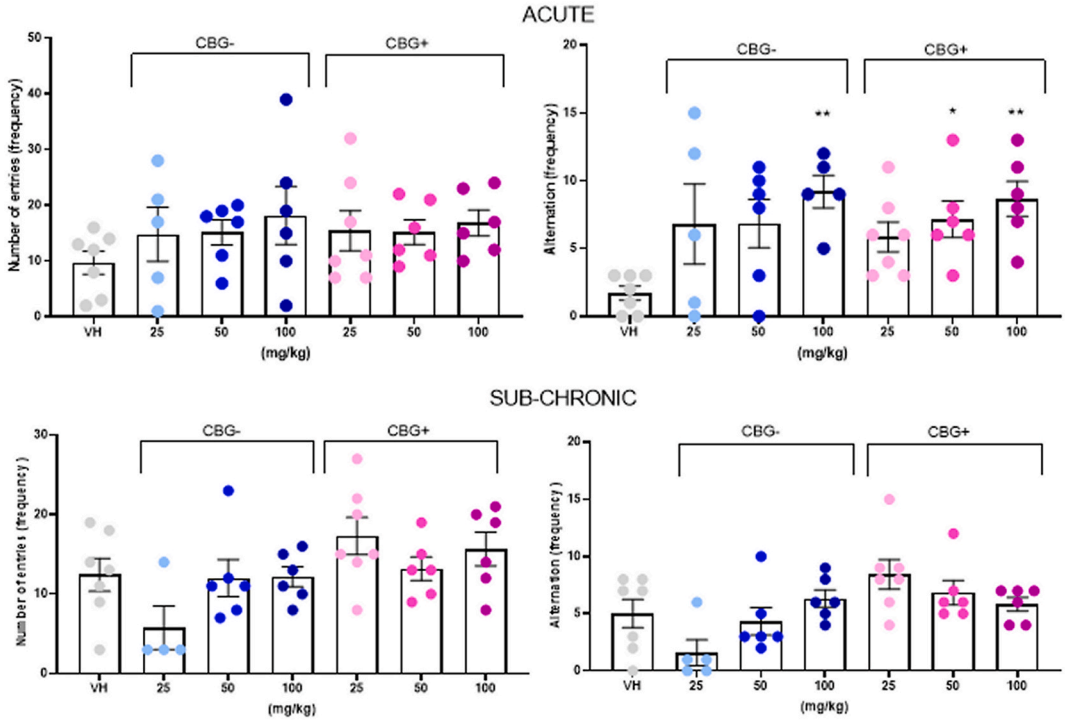

Fig. 4. Effects of CBG- and CBG+ formulas on mice short-term memory. Y-maze test was used to evaluated mice short-term working memory. The effects of CBG- and CBG+ formulas on number of arm entries and arm alternation are reported for acute treatment (up) and for sub-chronic treatment (down). Data are expressed as mean ± S.E.M. * = $p < 0.05$ and ** = $p < 0.01$ with one-way ANOVA followed by Dunnett's multiple comparison test.

maintained after repeated administrations.

3.4. Both formulas produce an increase in social activity in a dose-dependent manner

Lastly, we checked for a possible effect of the two formulas on social behavior in mice. Since it is known that CBD alone displays a prosocial effect in rodents [24,58], we wanted to assess if this effect is modulated by the presence of CBG.

We found that social behavior is significantly improved in mice treated with the CBG- formula. In male mice, spontaneous interaction with unfamiliar females was significantly increased and, conversely, time spent in non-social activities was decreased. We found this effect to be dose-dependent, and it was observed both in acute treatment and after 10 days formula administration (Supplementary Table 7), as reported in Fig. 5.

Treatment with CBG+ also displayed a prosocial effect compared to the vehicle group, in acute but not in sub-chronic treatment (Supplementary Table 8) (Fig. 5).

4. Discussion

The potential use of phytocannabinoids as therapeutic agents is a topic of great interest in the pharmacological field and, in particular, in neuropharmacology. Attention has focused, above all, on some, already partially well-characterized phytocannabinoids and which are present in greater quantities in *C. sativa*, such as cannabidiol (CBD). However, much work still remains to be done to understand the contribution of minor phytocannabinoids. The relevance of these compounds in the field of biomedical research has been neglected, leaving a gap in our understanding of how the consumption of *C. sativa* (and its derivatives) impacts biological, cognitive and behavioral dimensions. Phytocannabinoids have great potential in the therapeutic field and the studies conducted so far have also shown a good safety profile. For example, focusing on CBD, one of the best characterized *C. Sativa* components, its use as therapeutic agent has been recently demonstrated for a broad range of conditions, such as inflammation, epilepsy, cancer, neurodegenerative disease and others [14,15], with a safe profile both in humans and animals [47,48]. In recent years, preliminary studies have tried to shed light on the therapeutic benefits of cannabigerol (CBG), the parent molecule of THC and CBD. It has been noted that CBG acts against neuroinflammation in a rodent model of multiple sclerosis [38] and is effective in ameliorating conditions in other diseases characterized by inflammation, such as colitis [43] and psoriasis [59]. Much less is known about CBG effect on behavior. A first, preliminary survey of patients using CBG-rich cannabis preparations has reported self-assessed positive effects on conditions such as pain, depression and insomnia [47], but its potential effect coupled with other cannabinoids contained in *C. sativa* preparations is still uncertain. Recent reports have demonstrated that CBG administered in combination with CBD could have a positive effect on neuroinflammation associated to neurodegenerative disorders such as amyotrophic lateral sclerosis in an *in vitro* model [60]. However,

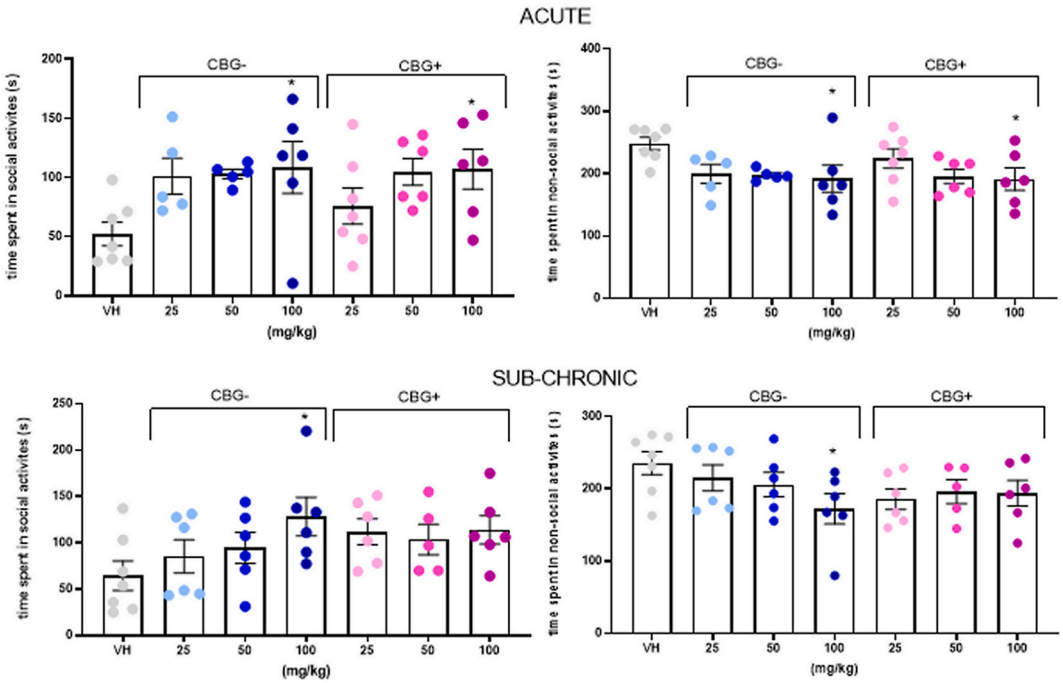

Fig. 5. Effects of CBG- and CBG+ formulas on mice social behavior. Reciprocal interaction test was used to evaluated mice sociability. The effects of CBG- and CBG+ formulas on time spent in social activities and time spent in non-social activities are reported for acute treatment (up) and for sub-chronic treatment (down). Data are expressed as mean ± S.E.M. * = $p < 0.05$ with one-way ANOVA followed by Dunnett's multiple comparison test.

the literature available concerning the effects on behavior and cognitive domains of preclinical models is scarce.

The purpose of this study was to characterize the behavioral effects of two novel non-psychotropic cannabinoid formulas, both containing relatively high percentages of CBD but differing mainly for CBG content, for this reason called CBG- and CBG+ formulas. To study this, the two formulas were tested *in vivo*, on wild type adult male mice, following oral administration. After acute or sub-chronic administration, a series of standardized behavioral tests were performed in different areas of interest (locomotor activity, anxiety, cognition and sociability). The results obtained from the various behavioral tests performed show that, changing the amount of CBG contained in the two formulas, we observe a completely different behavioral profile. In fact, following acute formulas administration, we observed that CBG- formula induced a significant increase in short-term working memory and also in social interaction; no significant effects were registered on both anxiety and motility. The acutely-administered CBG+ formula in addition to maintaining the beneficial effects induced by the CBG- formula, i.e. an improvement in cognitive performance and social interaction, it provoked novel behavioral effects, also inducing an anxiolytic effect and increased motility. Concerning sub-chronic administration, the situation is more complex. In fact, the results obtained show that the CBG- formula produced significant effects only in increasing social interaction; the CBG+ formula instead caused positive effects only on anxiety, maintaining its anxiolytic effects even in chronic administration. This result is in line with what Mendiguren et al. [61] found administering CBG in Sprague-Dawley rats. The authors report that a single 10 mg/kg dose via intraperitoneal injection had an anxiolytic effect, acting via the serotonergic pathway. Indeed, the anxiolytic effect could be explained by the CBG's action on 5-HT1A receptor, for which it is a moderately potent antagonist [62]. Similarly, Zagzoog et al. [45] previously reported that CBG (10 mg/kg) displayed an anxiolytic effect in mice, assessed via the increase in time spent in the central area of the open field test arena. However, contrary to these results, Zhou et al. [63] reported that acutely administered CBG (1–60 mg/kg) did not ameliorate stress-provoked anxiety-related behaviors. Additionally, it is to note that the differences seen between the behavioral results from the acute and sub-chronic treatment could be attributed to a pharmacodynamic tolerance mechanism. Chronic *in vivo* treatment with cannabinoids induces substantial tolerance to their physiological and behavioral effects, which has been linked to a significant reduction in the ability of cannabinoid receptors to couple with G-proteins in the brain. The extent of these effects varies across different brain regions and is often, though not always, associated with a decrease in cannabinoid receptor binding. While the exact relationship between receptor desensitization and tolerance remains unclear, these mechanisms likely contribute to the diminished response to cannabinoid agonists and the development of tolerance [64] which could possibly explain the behavioral differences between the two treatments.

Given this data from the literature, there are no conclusive indication about CBG's precise effects and mechanism of action on rodent behavior, but it is possible to speculate that the anxiolytic effects observed after CBG administration, along with its reported anti-inflammatory effects, are likely mediated via mechanisms unrelated to cannabinoid signaling [45], such as the PPARγ pathway [37], α2-adrenoceptor [65], 5HT1A receptors [62,63], and transient receptor potential (TRP) channels [65]. Regarding possible mechanisms of action through which CBD may exert its effects on behavior, research has specifically highlighted the role of the oxytocin pathway in enhancing social behavior, a process that appears to be influenced by the endocannabinoid system [66]. Our previous work [58] demonstrated that mice treated with *Cannabis sativa* oil, containing different types of phytocannabinoids and in particular CBD, exhibited increased social behavior compared to controls, an effect that was diminished when co-treated with an oxytocin antagonist. Additionally, *Cannabis sativa* oil treatment resulted in elevated oxytocin mRNA levels in the hypothalamus and a reduction in oxytocin receptor mRNA, suggesting a down-regulation mechanism triggered by robust oxytocin release and receptor activation. This mechanism may explain the behavioral effects observed with the compounds tested in this study, although further research is needed to specifically test this hypothesis.

The previous literature and the data obtained in this study suggest that CBG may play an important role in the modulation of behaviors such as locomotor activity, anxiety, memory and sociability. However, the present study reports some limitations that must be addressed. The main consideration needing to be acknowledged regards the composition of the CBG- and CBG+ formulas. Indeed, the formulas used in the present study are constituted by a mixture of phytocannabinoids – namely CBG, CBD and minor proportions of other cannabinoids, such as CBC – aside from terpenes and flavonoids. We cannot rule out the hypothesis that the unique behavioral profiles that emerged following the administration of the two formulas could be attributed to the synergistic action of the various phytocannabinoids and other compounds present in the mixture, albeit in smaller quantities. In this regard, some authors report the existence of an "entourage effect" – namely, a synergistic action of phytocannabinoids and the numerous terpenes present in the *C. sativa* plant, that would lead to different biological effects than the ones provoked by the use of phytocannabinoids alone [24,67]. In addition, it is worth mentioning that the formulas used present a non-negligible amount of cannabichromene (CBC) (8.0 % and 6.3 % of total mixture of CBG- and CBG+ formula, respectively), a major *C. sativa* phytocompound displaying potent biological effects [15]. Indeed, it has been shown that CBC displays anti-depressant like properties that could contribute to the antidepressant effect associated with *Cannabis* intake [68]. Moreover, CBC has been found to have a positive effect on mice adult neural stem/progenitor cells, that could be related to the CBC's known anti-inflammatory effect [69,70], acting via inhibition of glial cells to promote neurogenesis [71]. These findings show that CBC display a profound effect on the central nervous system, and its possible mechanism of action and biological effects should be addressed to further clarify the role of this phytocannabinoids in *C. sativa*-based extracts.

Lastly, this study serves as an initial exploration into the behavioral effects of various cannabinoid formulations, focusing exclusively on male animals to prevent estrous cycle-related behavioral fluctuations from confounding the results. While this approach helps ensure consistency in the initial data, it also limits the generalizability of the findings, as it overlooks potential sex-specific responses. Addressing this limitation in future studies by including female mice is essential for a more comprehensive understanding of cannabinoid effects across both sexes.

5. Conclusions

In conclusion, the study reported here underlines the importance of deepening knowledge on the possible effects of minor phytocannabinoids, used both alone and in combination, for a potential future therapeutic use. Further evaluations are needed to assess the effects of phytocannabinoids combinations on preclinical models. Our observations advocate for further research on the use of *C. sativa*'s different compounds, as the combination of distinct cannabinoids could lead to the development of new multi-targeted drugs with unique pharmacological profiles that could be tailored for different patient's specific needs.

CRediT authorship contribution statement

Marinella Carone: Writing – original draft, Investigation, Formal analysis. **Marika Premoli:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Sara Anna Bonini:** Writing – review & editing, Validation, Supervision, Investigation, Data curation, Conceptualization. **Rozana Latsi:** Investigation, Formal analysis. **Giuseppina Maccarinelli:** Investigation. **Maurizio Memo:** Writing – review & editing, Validation, Project administration, Funding acquisition, Data curation, Conceptualization.

Institutional review board statement

The animal study protocol was approved by the Animal care and use Committee of the University of Brescia and by the Italian Ministry of Health (protocol code 211B5.38, authorization n. 381/2019, approved May 20, 2019).

Data availability statement

Data is contained within the article.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bonini SA is Heliyon Associate Editor. The other 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e39938.

References

[1] F. Pellati, V. Borgonetti, V. Brighenti, M. Biagi, S. Benvenuti, L. Corsi, Cannabis sativa L. And nonpsychoactive cannabinoids: their chemistry and role against oxidative stress, inflammation, and cancer, BioMed Res. Int. 2018 (2018) 1691428, https://doi.org/10.1155/2018/1691428.
[2] G. Appendino, G. Chianese, O. Taglialatela-Scafati, Cannabinoids: occurrence and medicinal chemistry, Curr. Med. Chem. 18 (7) (2011) 1085–1099, https://doi.org/10.2174/092986711794940888.
[3] M.N. Tahir, F. Shahbazi, S. Rondeau-Gagne, J.F. Trant, The biosynthesis of the cannabinoids, J Cannabis Res 3 (1) (2021) 7, https://doi.org/10.1186/s42238-021-00062-4.
[4] S. Chandra, H. Lata, M. Elsohly, Cannabis sativa L. - botany and biotechnology (2017) 1–474, https://doi.org/10.1007/978-3-319-54564-6.
[5] S.D. Skaper, V. Di Marzo, Endocannabinoids in nervous system health and disease: the big picture in a nutshell, Philos. Trans. R. Soc. Lond. B Biol. Sci. 367 (1607) (2012) 3193–3200, https://doi.org/10.1098/rstb.2012.0313.
[6] D. An, S. Peigneur, L.A. Hendrickx, J. Tytgat, Targeting cannabinoid receptors: current status and prospects of natural products, Int. J. Mol. Sci. 21 (14) (2020), https://doi.org/10.3390/ijms21145064.
[7] F. Espejo-Porras, J. Fernández-Ruiz, R.G. Pertwee, R. Mechoulam, C. García, Motor effects of the non-psychotropic phytocannabinoid cannabidiol that are mediated by 5-HT1A receptors, Neuropharmacology 75 (2013) 155–163, https://doi.org/10.1016/j.neuropharm.2013.07.024.
[8] E. Landucci, D.E. Pellegrini-Giampietro, A. Gianoncelli, G. Ribaudo, Cannabidiol preferentially binds TRPV2: a novel mechanism of action, Neural Regen Res 17 (12) (2022) 2693–2694, https://doi.org/10.4103/1673-5374.335821.
[9] S. Elsaid, S. Kloiber, B. Le Foll, Effects of cannabidiol (CBD) in neuropsychiatric disorders: a review of pre-clinical and clinical findings, Prog Mol Biol Transl Sci 167 (2019) 25–75, https://doi.org/10.1016/bs.pmbts.2019.06.005.
[10] M.S. Garcia-Gutierrez, F. Navarrete, A. Gasparyan, A. Austrich-Olivares, F. Sala, J. Manzanares, Cannabidiol: a potential new alternative for the treatment of anxiety, depression, and psychotic disorders, Biomolecules 10 (11) (2020), https://doi.org/10.3390/biom10111575.
[11] J.W. Skelley, C.M. Deas, Z. Curren, J. Ennis, Use of cannabidiol in anxiety and anxiety-related disorders, J Am Pharm Assoc (2003) 60 (1) (2020) 253–261, https://doi.org/10.1016/j.japh.2019.11.008.
[12] N. Assareh, A. Gururajan, C. Zhou, J.L. Luo, R.C. Kevin, J.C. Arnold, Cannabidiol disrupts conditioned fear expression and cannabidiolic acid reduces trauma-induced anxiety-related behaviour in mice, Behav. Pharmacol. 31 (6) (2020) 591–596, https://doi.org/10.1097/FBP.0000000000000565.

[13] P.A. Melas, M. Scherma, W. Fratta, C. Cifani, P. Fadda, Cannabidiol as a potential treatment for anxiety and mood disorders: molecular targets and epigenetic insights from preclinical research, Int. J. Mol. Sci. 22 (4) (2021), https://doi.org/10.3390/ijms22041863.
[14] M. Premoli, F. Aria, S.A. Bonini, G. Maccarinelli, A. Gianoncelli, S. Della Pina, S. Tambaro, M. Memo, A. Mastinu, Cannabidiol: recent advances and new insights for neuropsychiatric disorders treatment, Life Sci. 224 (2019) 120–127, https://doi.org/10.1016/j.lfs.2019.03.053.
[15] J.H. Khalsa, G. Bunt, K. Blum, S.B. Maggirwar, M. Galanter, M.N. Potenza, Review: cannabinoids as medicinals, Curr Addict Rep 9 (4) (2022) 630–646, https://doi.org/10.1007/s40429-022-00438-3.
[16] A.R. Müller, B. den Hollander, P.M. van de Ven, K.C.B. Roes, L. Geertjens, H. Bruining, C.D.M. van Karnebeek, F.E. Jansen, M.C.Y. de Wit, L.W. Ten Hoopen, A. B. Rietman, B. Dierckx, F.A. Wijburg, E. Boot, M.M.G. Brands, A.M. van Eeghen, Cannabidiol (Epidyolex(R)) for severe behavioral manifestations in patients with tuberous sclerosis complex, mucopolysaccharidosis type III and fragile X syndrome: protocol for a series of randomized, placebo-controlled N-of-1 trials, BMC Psychiatr. 24 (1) (2024) 23, https://doi.org/10.1186/s12888-023-05422-3.
[17] N.F. Parrella, A.T. Hill, P.G. Enticott, P. Barhoun, I.S. Bower, T.C. Ford, A systematic review of cannabidiol trials in neurodevelopmental disorders, Pharmacol. Biochem. Behav. 230 (2023) 173607, https://doi.org/10.1016/j.pbb.2023.173607.
[18] O.P. Omotayo, Y. Lemmer, S. Mason, A narrative review of the therapeutic and remedial prospects of cannabidiol with emphasis on neurological and neuropsychiatric disorders, J Cannabis Res 6 (1) (2024) 14, https://doi.org/10.1186/s42238-024-00222-2.
[19] R.M. Vitale, F.A. Iannotti, P. Amodeo, The (Poly)Pharmacology of cannabidiol in neurological and neuropsychiatric disorders: molecular mechanisms and targets, Int. J. Mol. Sci. 22 (9) (2021), https://doi.org/10.3390/ijms22094876.
[20] J. Zieba, D. Sinclair, T. Sebree, M. Bonn-Miller, D. Gutterman, S. Siegel, T. Karl, Cannabidiol (CBD) reduces anxiety-related behavior in mice via an FMRP-independent mechanism, Pharmacol. Biochem. Behav. 181 (2019) 93–100, https://doi.org/10.1016/j.pbb.2019.05.002.
[21] F. Kreilaus, M. Przybyla, L. Ittner, T. Karl, Cannabidiol (CBD) treatment improves spatial memory in 14-month-old female TAU58/2 transgenic mice, Behav. Brain Res. 425 (2022) 113812, https://doi.org/10.1016/j.bbr.2022.113812.
[22] S.H. Shrader, N. Mellen, J. Cai, G.N. Barnes, Z.H. Song, Cannabidiol is a behavioral modulator in BTBR mouse model of idiopathic autism, Front. Neurosci. 18 (2024) 1359810, https://doi.org/10.3389/fnins.2024.1359810.
[23] A. Manduca, V. Buzzelli, A. Rava, A. Feo, E. Carbone, S. Schiavi, B. Peruzzi, V. D'Oria, M. Pezzullo, A. Pasquadibisceglie, F. Polticelli, V. Micale, M. Kuchar, V. Trezza, Cannabidiol and positive effects on object recognition memory in an in vivo model of Fragile X Syndrome: obligatory role of hippocampal GPR55 receptors, Pharmacol. Res. 203 (2024) 107176, https://doi.org/10.1016/j.phrs.2024.107176.
[24] A. Mastinu, R. Ascrizzi, G. Ribaudo, S.A. Bonini, M. Premoli, F. Aria, G. Maccarinelli, A. Gianoncelli, G. Flamini, L. Pistelli, M. Memo, Prosocial effects of nonpsychotropic cannabis sativa in mice, Cannabis Cannabinoid Res 7 (2) (2022) 170–178, https://doi.org/10.1089/can.2021.0017.
[25] J. Staben, M. Koch, K. Reid, J. Muckerheide, L. Gilman, F. McGuinness, S. Kiesser, I.W.H. Oswald, K.A. Koby, T.J. Martin, J.S. Kaplan, Cannabidiol and cannabis-inspired terpene blends have acute prosocial effects in the BTBR mouse model of autism spectrum disorder, Front. Neurosci. (2023), https://doi.org/10.3389/fnins.2023.1185737.
[26] E. Jones, S. Vlachou, A critical review of the role of the cannabinoid compounds delta(9)-tetrahydrocannabinol (Delta(9)-THC) and cannabidiol (CBD) and their combination in multiple sclerosis treatment, Molecules 25 (21) (2020), https://doi.org/10.3390/molecules25214930.
[27] A. Feliú, M. Moreno-Martet, M. Mecha, F.J. Carrillo-Salinas, E. de Lago, J. Fernández-Ruiz, C. Guaza, A Sativex((R)) -like combination of phytocannabinoids as a disease-modifying therapy in a viral model of multiple sclerosis, Br. J. Pharmacol. 172 (14) (2015) 3579–3595, https://doi.org/10.1111/bph.13159.
[28] Y. Javadzadeh, A. Santos, M.S. Aquilino, S. Mylvaganam, K. Urban, P.L. Carlen, Cannabidiol exerts anticonvulsant effects alone and in combination with delta (9)-THC through the 5-ht1a receptor in the neocortex of mice, Cells 13 (6) (2024).
[29] F. Calapai, L. Cardia, E. Esposito, I. Ammendolia, C. Mondello, Giudice R. Lo, et al., Pharmacological aspects and biological effects of cannabigerol and its synthetic derivatives, Evid Based Complement Alternat Med 2022 (2022) 3336516, https://doi.org/10.3390/cells13060466.
[30] N.M. Kogan, Y. Lavi, L.M. Topping, R.O. Williams, F.E. McCann, Z. Yekhtin, M. Feldmann, R. Gallily, R. Mechoulam, Novel CBG derivatives can reduce inflammation, pain and obesity, Molecules 26 (18) (2021), https://doi.org/10.3390/molecules26185601.
[31] A. Jastrzab, I. Jarocka-Karpowicz, E. Skrzydlewska, The origin and biomedical relevance of cannabigerol, Int. J. Mol. Sci. 23 (14) (2022), https://doi.org/10.3390/ijms23147929.
[32] R. Nachnani, W.M. Raup-Konsavage, K.E. Vrana, The pharmacological case for cannabigerol, J. Pharmacol. Exp. Therapeut. 376 (2) (2021) 204–212, https://doi.org/10.1124/jpet.120.000340.
[33] A. Gugliandolo, F. Pollastro, G. Grassi, P. Bramanti, E. Mazzon, In vitro model of neuroinflammation: efficacy of cannabigerol, a non-psychoactive cannabinoid, Int. J. Mol. Sci. 19 (7) (2018), https://doi.org/10.3390/ijms19071992.
[34] V. di Giacomo, A. Chiavaroli, G. Orlando, A. Cataldi, M. Rapino, V. Di Valerio, S. Leone, L. Brunetti, L. Menghini, L. Recinella, C. Ferrante, Neuroprotective and neuromodulatory effects induced by cannabidiol and cannabigerol in rat hypo-E22 cells and isolated hypothalamus, Antioxidants 9 (1) (2020), https://doi.org/10.3390/antiox9010071.
[35] J. Díaz-Alonso, J. Paraíso-Luna, C. Navarrete, C. Del Río, I. Cantarero, B. Palomares, J. Aguareles, J. Fernández-Ruiz, M. Luz Bellido, F. Pollastro, G. Appendino, M. A Calzado, I. Galve-Roperh, E. Muñoz, VCE-003.2, a novel cannabigerol derivative, enhances neuronal progenitor cell survival and alleviates symptomatology in murine models of Huntington's disease, Sci. Rep. 6 (2016) 29789, https://doi.org/10.1038/srep29789.
[36] S. Burgaz, C. Garcia, M. Gomez-Canas, E. Munoz, J. Fernandez-Ruiz, Development of an oral treatment with the PPAR-gamma-acting cannabinoid VCE-003.2 against the inflammation-driven neuronal deterioration in experimental Parkinson's disease, Molecules 24 (15) (2019), https://doi.org/10.3390/molecules24152702.
[37] A.G. Granja, F. Carrillo-Salinas, A. Pagani, M. Gomez-Canas, R. Negri, C. Navarrete, M. Mecha, L. Mestre, B. L Fiebich, I. Cantarero, M.A. Calzado, M.L. Bellido, J. Fernandez-Ruiz, G. Appendino, C. Guaza, E. Muñoz, A cannabigerol quinone alleviates neuroinflammation in a chronic model of multiple sclerosis, J. Neuroimmune Pharmacol. 7 (4) (2012) 1002–1016, https://doi.org/10.1007/s11481-012-9399-3.
[38] S. Fleisher-Berkovich, Y. Ventura, M. Amoyal, A. Dahan, V. Feinshtein, L. Alfahel, A. Israelson, N. Bernstein, J. Gorelick, S. Ben-Shabat, Therapeutic potential of phytocannabinoid cannabigerol for multiple sclerosis: modulation of microglial activation in vitro and in vivo, Biomolecules 13 (2) (2023), https://doi.org/10.3390/biom13020376.
[39] E. Pagano, F.A. Iannotti, F. Piscitelli, B. Romano, G. Lucariello, T. Venneri, V. Di Marzo, A.A. Izzo, F. Borrelli, Efficacy of combined therapy with fish oil and phytocannabinoids in murine intestinal inflammation, Phytother Res. 35 (1) (2021) 517–529, https://doi.org/10.1002/ptr.6831.
[40] F. Calapai, L. Cardia, E. Esposito, I. Ammendolia, C. Mondello, R. Lo Giudice, S. Gangemi, G. Calapai, C. Mannucci, Pharmacological aspects and biological effects of cannabigerol and its synthetic derivatives, Evid Based Complement Alternat Med (2022), https://doi.org/10.1155/2022/3336516.
[41] M.G. Cascio, L.A. Gauson, L.A. Stevenson, R.A. Ross, R.G. Pertwee, Evidence that the plant cannabinoid cannabigerol is a highly potent alpha2-adrenoceptor agonist and moderately potent 5HT1A receptor antagonist, Br. J. Pharmacol. 159 (1) (2010) 129–141, https://doi.org/10.1111/j.1476-5381.2009.00515.x.
[42] Y. Wen, Z. Wang, R. Zhang, Y. Zhu, G. Lin, R. Li, J. Zhang, The antinociceptive activity and mechanism of action of cannabigerol, Biomed. Pharmacother. 158 (2023) 114163, https://doi.org/10.1016/j.biopha.2022.114163.
[43] F. Borrelli, I. Fasolino, B. Romano, R. Capasso, F. Maiello, D. Coppola, P. Orlando, G. Battista, E. Pagano, V. Di Marzo, A.A. Izzo, Beneficial effect of the non-psychotropic plant cannabinoid cannabigerol on experimental inflammatory bowel disease, Biochem. Pharmacol. 85 (9) (2013) 1306–1316, https://doi.org/10.1016/j.bcp.2013.01.017.
[44] D.G. Couch, H. Maudslay, B. Doleman, J.N. Lund, S.E. O'Sullivan, The use of cannabinoids in colitis: a systematic review and meta-analysis, Inflamm. Bowel Dis. 24 (4) (2018) 680–697, https://doi.org/10.1093/ibd/izy014.
[45] A. Zagzoog, K.A. Mohamed, H.J.J. Kim, E.D. Kim, C.S. Frank, T. Black, P.D. Jadhav, L.A. Holbrook, R.B. Laprairie, In vitro and in vivo pharmacological activity of minor cannabinoids isolated from Cannabis sativa, Sci. Rep. 10 (1) (2020) 20405, https://doi.org/10.1038/s41598-020-77175-y.
[46] E.A. Formukong, A.T. Evans, F.J. Evans, Analgesic and antiinflammatory activity of constituents of Cannabis sativa L, Inflammation 12 (4) (1988) 361–371, https://doi.org/10.1007/BF00915771.

[47] E.B. Russo, C. Cuttler, Z.D. Cooper, A. Stueber, V.L. Whiteley, M. Sexton, Survey of patients employing cannabigerol-predominant cannabis preparations: perceived medical effects, adverse events, and withdrawal symptoms, Cannabis Cannabinoid Res 7 (5) (2022) 706–716, https://doi.org/10.1089/can.2021.0058.
[48] D.K. Khajuria, V. Karuppagounder, I. Nowak, D.E. Sepulveda, G.S. Lewis, C.C. Norbury, W.M. Raup-Konsavage, K.E. Vrana, F. Kamal, R.A. Elbarbary, Cannabidiol and cannabigerol, nonpsychotropic cannabinoids, as analgesics that effectively manage bone fracture pain and promote healing in mice, J. Bone Miner. Res. 38 (11) (2023) 1560–1576, https://doi.org/10.1002/jbmr.4902.
[49] C. Echeverry, G. Prunell, C. Narbondo, V.S. de Medina, X. Nadal, M. Reyes-Parada, C. Scorza, A comparative in vitro study of the neuroprotective effect induced by cannabidiol, cannabigerol, and their respective acid forms: relevance of the 5-HT(1A) receptors, Neurotox. Res. 39 (2) (2021) 335–348, https://doi.org/10.1007/s12640-020-00277-y.
[50] T.T. Lah, M. Novak, M.A. Pena Almidon, O. Marinelli, B. Žvar Baškovič, B. Majc, M. Mlinar, R. Bošnjak, B. Breznik, R. Zomer, M. Nabissi, Cannabigerol is a potential therapeutic agent in a novel combined therapy for glioblastoma, Cells 10 (2) (2021), https://doi.org/10.3390/cells10020340.
[51] C.L. Robaina Cabrera, S. Keir-Rudman, N. Horniman, N. Clarkson, C. Page, The anti-inflammatory effects of cannabidiol and cannabigerol alone, and in combination, Pulm. Pharmacol. Ther. 69 (2021) 102047, https://doi.org/10.1016/j.pupt.2021.102047.
[52] F. Aria, S.A. Bonini, V. Cattaneo, M. Premoli, A. Mastinu, G. Maccarinelli, M. Memo, Brain structural and functional alterations in mice prenatally exposed to LPS are only partially rescued by anti-inflammatory treatment, Brain Sci. 10 (9) (2020), https://doi.org/10.3390/brainsci10090620.
[53] M. Pucci, F. Aria, M. Premoli, G. Maccarinelli, A. Mastinu, S.A. Bonini, M. Memo, D. Uberti, G. Abate, Methylglyoxal affects cognitive behaviour and modulates RAGE and Presenilin-1 expression in hippocampus of aged mice, Food Chem. Toxicol. 158 (2021 Dec) 112608, https://doi.org/10.1016/j.fct.2021.112608.
[54] A.K. Kraeuter, P.C. Guest, Z. Sarnyai, The elevated plus maze test for measuring anxiety-like behavior in rodents, Methods Mol. Biol. 1916 (2019) 69–74, https://doi.org/10.1007/978-1-4939-8994-2_4.
[55] A.A. Walf, C.A. Frye, The use of the elevated plus maze as an assay of anxiety-related behavior in rodents, Nat. Protoc. 2 (2) (2007) 322–328, https://doi.org/10.1038/nprot.2007.44.
[56] A.K. Kraeuter, P.C. Guest, Z. Sarnyai, The Y-maze for assessment of spatial working and reference memory in mice, Methods Mol. Biol. 1916 (2019) 105–111, https://doi.org/10.1007/978-1-4939-8994-2_10.
[57] E. Borsani, F. Bonomini, S.A. Bonini, M. Premoli, G. Maccarinelli, L. Giugno, A. Mastinu, F. Aria, M. Memo, R. Rezzani, Role of melatonin in autism spectrum disorders in a male murine transgenic model: study in the prefrontal cortex, J. Neurosci. Res. 100 (3) (2022) 780–797, https://doi.org/10.1002/jnr.24997.
[58] M. Premoli, M. Carone, A. Mastinu, G. Maccarinelli, F. Aria, E. Mac Sweeney, M. Memo, S.A. Bonini, Cannabis sativa oil promotes social interaction and ultrasonic communication by acting on oxytocin pathway, Cannabis Cannabinoid Res 27 (2024 May), https://doi.org/10.1089/can.2024.0062.
[59] E. Perez, J.R. Fernandez, C. Fitzgerald, K. Rouzard, M. Tamura, C. Savile, In vitro and clinical evaluation of cannabigerol (CBG) produced via yeast biosynthesis: a cannabinoid with a broad range of anti-inflammatory and skin health-boosting properties, Molecules 27 (2) (2022), https://doi.org/10.3390/molecules27020491.
[60] C. Rodríguez-Cueto, I. Santos-García, L. García-Toscano, F. Espejo-Porras, M. Bellido, J. Fernández-Ruiz, E. Muñoz, E. de Lago, Neuroprotective effects of the cannabigerol quinone derivative VCE-003.2 in SOD1G93A transgenic mice, an experimental model of amyotrophic lateral sclerosis, Biochem. Pharmacol. 157 (2018 Nov) 217–226, https://doi.org/10.1016/j.bcp.2018.07.049.
[61] A. Mendiguren, E. Aostri, I. Rodilla, I. Pujana, E. Noskova, J. Pineda, Cannabigerol modulates alpha(2)-adrenoceptor and 5-HT(1A) receptor-mediated electrophysiological effects on dorsal raphe nucleus and locus coeruleus neurons and anxiety behavior in rat, Front. Pharmacol. 14 (2023) 1183019, https://doi.org/10.3389/fphar.2023.1183019.
[62] M.G. Cascio, L.A. Gauson, L.A. Stevenson, R.A. Ross, R.G. Pertwee, Evidence that the plant cannabinoid cannabigerol is a highly potent alpha2-adrenoceptor agonist and moderately potent 5HT1A receptor antagonist, Br. J. Pharmacol. 159 (1) (2010) 129–141, https://doi.org/10.1111/j.1476-5381.2009.00515.x.
[63] C. Zhou, N. Assareh, J.C. Arnold, The cannabis constituent cannabigerol does not disrupt fear memory processes or stress-induced anxiety in mice, Cannabis Cannabinoid Res 7 (3) (2022) 294–303, https://doi.org/10.1089/can.2021.0027.
[64] S.R. Childers, Activation of G-proteins in brain by endogenous and exogenous cannabinoids. AAPS J., 8, E112-E117. doi: 10.1208/aapsj080113.
[65] L. De Petrocellis, A. Ligresti, A.S. Moriello, M. Allarà, T. Bisogno, S. Petrosino, C.G. Stott, V. Di Marzo, Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes, Br. J. Pharmacol. 163 (7) (2011) 1479–1494, https://doi.org/10.1111/j.1476-5381.2010.01166.x.
[66] D. Wei, D. Lee, C.D. Cox, C.A. Karsten, O. Peñagarikano, D.H. Geschwind, C.M. Gall, D. Piomelli, Endocannabinoid signaling mediates oxytocin-driven social reward, Proc. Natl. Acad. Sci. U. S. A. 112 (45) (2015 Nov 10) 14084–14089, https://doi.org/10.1073/pnas.1509795112.
[67] E.B. Russo, The case for the entourage effect and conventional breeding of clinical cannabis: No "strain," No gain, Front. Plant Sci. 9 (2018) 1969, https://doi.org/10.3389/fpls.2018.01969.
[68] A.T. El-Alfy, K. Ivey, K. Robinson, S. Ahmed, M. Radwan, D. Slade, I. Khan, M. ElSohly, S. Ross, Antidepressant-like effect of delta9-tetrahydrocannabinol and other cannabinoids isolated from Cannabis sativa L, Pharmacol. Biochem. Behav. 95 (4) (2010) 434–442, https://doi.org/10.1016/j.pbb.2010.03.004.
[69] A.A. Izzo, R. Capasso, G. Aviello, F. Borrelli, B. Romano, F. Piscitelli, L. Gallo, F. Capasso, P. Orlando, V. Di Marzo, Inhibitory effect of cannabichromene, a major non-psychotropic cannabinoid extracted from Cannabis sativa, on inflammation-induced hypermotility in mice, Br. J. Pharmacol. 166 (4) (2012) 1444–1460, https://doi.org/10.1111/j.1476-5381.2012.01879.x.
[70] P.W. Wirth, E.S. Watson, M. ElSohly, C.E. Turner, J.C. Murphy, Anti-inflammatory properties of cannabichromene, Life Sci. 26 (23) (1980) 1991–1995, https://doi.org/10.1016/0024-3205(80)90631-1.
[71] N. Shinjyo, V. Di Marzo, The effect of cannabichromene on adult neural stem/progenitor cells, Neurochem. Int. 63 (5) (2013) 432–437, https://doi.org/10.1016/j.neuint.2013.08.002.