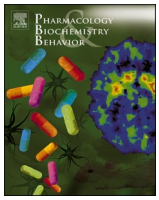
Pharmacology, Biochemistry and Behavior 249 (2025) 173971

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh



Purified cannabidiol leads to improvement of severe treatment-resistant behavioral symptoms in children with autism spectrum disorder

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ARTICLE INFO

Keywords:
Autism spectrum disorder
Behavior disturbances
Cannabidiol
Sleep
Treatment

ABSTRACT

Objective: The aim of our study was to evaluate the efficacy and safety of purified cannabidiol as an add-on medication in pediatric patients with autism spectrum disorder (ASD) associated with treatment resistant repetitive behaviors, behavior disorders, and intellectual disability and unresponsive to conventional medications and behavioral interventions.

Material and methods: A prospective, observational, before-and-after study was conducted including 20 patients with severe ASD who initiated treatment with purified CBD. Patients were evaluated using different scales at baseline and at three-month intervals during followup.

Results: The median total CBD dose was 363.5 mg (range, 100–700), and the median follow-up was 11 months (range, 6–12). As to the primary outcome evaluating symptoms reported by parents, improvement in at least one was observed after CBD initiation in 18 patients (90 %) and no improvement in two (10 %) (1 worsening, 1 no response). In the responders, 83.5 % ($n = 76$) of all reported symptoms improved. Regarding the secondary outcomes based on the assessment with different scales, improvement of around 30 % was found in irritability, social withdrawal, hyperactivity. Restricted and repetitive behavior improved in nine (50 %), while no changes were seen in seven (38.8 %). Sleep patterns were found to be slightly improved. Adverse effects were reported in 13 patients (65 %), mainly consisting of increased irritability and decreased appetite, but were mild or moderate and transient in all. In 40 % of the children, concomitant medication could be reduced or partially discontinued.

Conclusion: Our results suggest that treatment with purified CBD is effective and safe and could benefit patients with severe ASD by improving some of the core symptoms, including repetitive behaviors and social interaction, as well as associated comorbidities. The families considered the quality of life to have improved.

Key messages

- Almost half of the children with autism spectrum disorder and exhibiting disruptive behavior fail to respond to behavioral and medical interventions.
- Cannabidiol may benefit patients with severe autism by improving some of the core symptoms, including repetitive behaviors and social interaction, as well as associated comorbidities, such as disruptive behaviors and sleep disorders.
- Families observe a positive impact of cannabidiol on the daily management of the child, family dynamics, stress levels, and overall quality of life.
- Conventional medications, often associated with severe side effects such as weight gain, can be reduced or discontinued in a large proportion of the patients.

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by core symptoms that include persistent deficits in social communication and interaction, as well as restricted and repetitive patterns in behavior, interests, or activities (American Psychiatric Association, DSM-5, 2013). In addition, ASD is associated with heterogeneous non-core symptoms and comorbidities, both psychiatric and medical, such as social anxiety and oppositional defiant disorders,

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https://doi.org/10.1016/j.pbb.2025.173971
Received 3 December 2024; Received in revised form 26 January 2025; Accepted 14 February 2025
Available online 16 February 2025
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attention-deficit/hyperactivity, intellectual disability (ID), irritability, depression, seizures, gastrointestinal disorders, mitochondrial dysfunction, immune system abnormalities, and sleep disorders (Bauman, 2010; Masi et al., 2017; Mostafavi and Gaitanis, 2020). Severity is defined based on social communication impairments and restricted, repetitive patterns of behavior. The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (American Psychiatric Association, DSM-5) has incorporated severity levels for ASD based on the support needed, including requires support (Level 1), requires substantial support (Level 2), and requires very substantial support (Level 3) (American Psychiatric Association, DSM-5).

There is no established pharmacological treatment for ASD core signs and symptoms. Treatment is aimed at minimizing the core features and comorbidities, achieving functional independence, improving quality of life, and relieving family distress through behavioral, occupational, and speech therapies (Anagnostou, 2018). Conventional medical treatments to manage comorbid irritability and aggressive behavior include various psychotropic medications such as atypical antipsychotics, selective serotonin reuptake inhibitors (SSRIs), stimulants, and anxiolytics (Adler et al., 2015). Currently, only risperidone and aripiprazole have been approved by the U.S. Food and Drug Administration from the ages of 5 and 6 years, respectively, to address irritability associated with ASD; however, they often lead to obesity and metabolic syndrome (Holdman et al., 2022; Wink et al., 2014; Goel et al., 2018).

Approximately 40 % of children diagnosed with ASD and exhibiting disruptive behavior fail to respond to these behavioral and medical interventions (Adler et al., 2015). The behavioral challenges may lead to increased social isolation of the patients and their families and make it harder to obtain benefits from intervention strategies. Consequently, a significant proportion of parents are turning to alternative treatments (Höfer et al., 2017), such as the use of cannabis-derived products. In the majority of cases, they are accessing these products without proper pharmacological controls, mainly due to insufficient medical and scientific evidence to substantiate their efficacy (Holdman et al., 2022).

Cannabis has historically been used for both recreational and medicinal purposes, demonstrating good results in conditions including epilepsy, pain, and anorexia (Russo, 2017). Among the many bioactive compounds of the plant, the cannabinoids delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the most important. THC has psychotropic effects, may cause anxiety, and affects appetite, cognitive function, and memory. In contrast, CBD lacks psychoactive properties, exhibits a relatively high toxicity threshold, and is considered as an anti-inflammatory agent, and may therefore be beneficial in epilepsy and psychiatric disorders (Agarwal et al., 2019; Russo, 2011; Stolar et al., 2022).

ASD is a highly complex condition with a pathophysiology that is still poorly understood. Nevertheless, emerging research has shown that the endocannabinoid system (eCB) is altered in different neuropsychological and neurodevelopmental diseases (Chakrabarti et al., 2015; Zamberletti et al., 2017; Zou et al., 2021). Children with ASD have been found to have lower peripheral eCB levels (Karhson et al., 2018; Aran et al., 2019a, 2019b). The eCB is a cell-signaling network that modulates several organ systems (Mostafavi and Gaitanis, 2020), and is considered to play a role in neurotransmission, synaptic excitation and inhibition, and brain plasticity. In addition, it has been shown to be involved in aspects related to social interaction, motor control, repetitive behaviors, emotional processing, learning, and memory (Zou et al., 2021).

Therefore, the eCB system has emerged as a promising target for the treatment of core and non-core symptoms as well as comorbidities of ASD. The combination of anxiolytic, anti-inflammatory, and immune-regulatory properties, together with a lack of psychoactive effects and favorable safety profile, makes CBD an interesting treatment option for children with ASD. Different CBD formulations have been used, including CBD-enriched, highly purified, or synthetic pharmaceutical grade CBD, depending on the psychotropic THC co-content.

The objective of our study was to evaluate the effectiveness and safety of purified CBD as an add-on medication in 20 pediatric patients with ASD associated with repetitive behaviors, behavior disorders, and intellectual disability and unresponsive to conventional medications and behavioral interventions.

2. Material and methods

A prospective, observational, before-and-after study was conducted including 20 patients with ASD and severe treatment-resistant behavioral symptoms who initiated treatment with purified CBD.

Children and adolescents (3 to 18 years of age) with a diagnosis of ASD according to the DSM-5 diagnostic criteria were included in the study if they met the following criteria:

- Severity Level 2 or 3 based the core symptoms of ASD including impaired social communication and restricted and repetitive behavior patterns according to the DSM-5.
- ID (specified/unspecified, if the patient's profile allowed assessment with formal testing), or classified according to DSM-5 as mild, moderate, severe, or profound ID.
- Language impairment (unintelligible “nonverbal” speech, use of single words or very short sentences) or without accompanying language impairment (whole sentence speech or fluent speech).
- Behavioral disorders and disruptive behavior with self- and hetero-aggressions occurring on an almost daily basis requiring caregiver restraint and antipsychotic drug treatment and that failed in monotherapy or in combination.
- Daily, frequent, and sustained (>10 min) repetitive behavior.
- Intense hyperactivity interfering with daily activities and medical treatment or behavioral interventions.

Patients with epilepsy were excluded as epilepsy has been shown to respond well to CBD therapy and this may thus be a possible confounder. Exclusion criteria were patients with ASD severity Level 1 without ID, metabolic or immunologic disease, heart disease, genetic disorders, infections and/or bleeding, allergy to any of the components of CBD oil, pregnancy, cannabis use 7 days prior to the start of the study, and families at high risk of non-compliance with the protocol.

2.1. Intervention

All patients received treatment with highly purified CBD (Convupidiol® oral cannabidiol solution). The product was evaluated and approved by the Argentine National Administration of Drugs, Foods, and Medical Devices (ANMAT). The starting dose was 2 mg/kg/day for children weighing ≤45 kg, and 5 mg/kg/day in children weighing >45 kg divided into 2 doses. Drug-sensitive children were started on 1 mg/kg/day. Subsequently, the dose was uptitrated in increments equal to the starting dose (i.e., 2 mg/kg/day or 1 mg/kg/day) until the patient's baseline condition improved markedly, lack of tolerance, or up to a maximum dose of 25 mg/kg/day or 700 mg was achieved. The starting dose was maintained for 2 weeks and at the third week it was progressively increased according to the evolution of the symptoms described by the parents in the interviews held by telephone every 7, 10, 15 days or during office visits, according to the study phase. CBD was administered as an add-on to any ongoing stable medication (antipsychotics, CNS stimulants, etc.) or behavioral therapy.

2.2. Assessments

The clinicopathological characteristics of study participants were recorded at baseline, including age, gender, weight, and comorbidities.

2.2.1. Primary outcome

As a primary outcome we compared patients who had changes in at least one of five of the most severe symptoms of ASD identified by the

parents in the home, recreational, and therapeutic environments affecting quality of life of the patient and their families before CBD initiation and after each 3 months on CBD treatment.

Whenever possible, both parents jointly selected the most severe main symptoms, determined their frequency, and completed the questionnaires for each evaluation. When this was not feasible, the mothers selected the symptoms and completed the questionnaires. During face-to-face or telephone follow-ups, the primary investigator (SF) evaluated each child, analyzed the parents' responses, processed the data, and conducted continuous follow-up from the beginning of the protocol.

To measure effectivity, changes were rated on a 6-point scale (1: much improved, >75 % reduction of the symptom/behavior frequency; 2: improved, 74–50 % frequency reduction; 3: slightly improved, 49–25 % frequency reduction; 4: unchanged, 24–0 % frequency reduction; 5: slightly worse; 6: much worse. A positive response was defined as scores of 1, 2, or 3.

2.2.2. Secondary outcomes

For secondary outcomes we evaluated changes on the following scales before CBD initiation and performed each 3 months on CBD treatment:

Repetitive Behaviors Scale-Revised (RBS-R): This scale was used to measure the severity of repetitive behavior in people with autism spectrum disorder and ID. The RBS-R consists of six subscales including: Stereotyped Behavior, Self-injurious Behavior, Compulsive Behavior, Routine Behavior, Sameness Behavior, and Restricted Behavior. The presence and frequency of the behaviors are rated on a 4-point scale (i.e., 0 = behavior does not occur, 1 = behavior occurs and is a mild problem, 2 = behavior occurs and is a moderate problem, 3 = behavior occurs and is a severe problem) (Bodfish et al., 2000; Martínez-González et al., 2021).

Maladaptive Behavior Domain of the Vineland Adaptive Behavior Scales-II (VABS-II): This questionnaire was used to assess internalizing, externalizing, and other challenging behaviors of the children. The items are rated on a 3-point scale (0 = never; 1 = sometimes; 2 = usually or often). The maladaptive behavior subscales and index raw scores correspond to a V score (1 to 24) determining three clinical levels: clinically significant (21 to 24), elevated (18 to 20), or average (1 to 17) (Sparrow et al., 2005).

Pediatric Sleep Clinical Global Impressions Scale severity (CGI-S): The CGI-S is a clinician-based questionnaire that was used to evaluate sleep patterns of the patients. Aspects of sleep included in the scale are (1) the child's ability to fall asleep and remain sleeping independently (e.g., apart from parents); (2) bedtime resistance; (3) sleep onset delay; (4) night awakening; (5) parental satisfaction with their child's current sleep patterns; (6) family functioning as affected by their child's current sleep patterns; and (7) clinician's overall concern with the child's sleep. The questions are rated by the clinician on a scale of 1 to 7, with 1 representing no concerns and 7 among the highest level of concerns (Malow et al., 2016).

Aberrant Behavior Checklist: The ABC is a five-factor scale comprising 58 items used to assess drug and other treatment effects on individuals with ID. Each behavior is scored on a 4-point scale from 0 (not a problem) to 4 (serious problem) (Aman et al., 1985). For our study we selected 3 of the 5 factors of the scale: factor I: Irritability, agitation, crying, factor II: lethargy/social withdrawal, and factor IV: hyperactivity/noncompliance.

Autism Family Experience Questionnaire (AFEQ): The AFEQ, a four-domain, 48-item questionnaire, was used to evaluate the experience of a child with autism, family life, child development, child symptoms (feelings and behavior) with a score from 1 (always) to 5 (never). A lower score (minimum 48) indicates a positive outcome and a higher score a poor outcome (maximum 240) (Leadbitter et al., 2018).

Parental Stress Scale (PSS): The PSS, an 18-item self-report scale, was used to evaluate stress experienced by parents. The scale considers positive and negative aspects of parenting with a 5-point scale representing strongly disagree, disagree, undecided, agree, strongly agree, with possible scores ranging from 18 to 90. Higher scores reflect more parental stress (Berry and Jones, 1995).

To evaluate tolerability and safety of CBD, the parents or caregivers of the patients filled out a daily standardized form of adverse effects (AE). Severe AE were those that led to treatment discontinuation or required a visit to the emergency department. Adherence was evaluated during office visits and by telephone every 7, 10, 15 days according to the study phase.

3. Results

3.1. General features

Between January and December 2023, 20 patients were enrolled in the study, of whom 17 (85 %) were male. The mean age ± standard deviation (SD) of the patients was 10 (±4.58) years. Median weight at baseline was 45 kg (IQR, 32–71) and mean weight was 53.8 (±31.6), ranging from 17 to 148 kg.

Based on the DSM-5 criteria for assessing severity in social communication and restricted and repetitive behaviors, six patients (30 %) were classified as Level 2, and 14 patients (70 %) as Level 3.

All 20 patients presented with associated ID. According to the DSM-5, two patients (10 %) had mild ID, nine (45 %) had moderate ID, eight (40 %) had severe ID, and one patient (5 %) had profound ID.

Eighteen patients (90 %) had language involvement, of whom 10 patients (50 %) were nonverbal. Of the verbal patients, five (25 %) expressed single words and three (15 %) short sentences. Two patients (10 %) without language involvement had fluent speech, but with disorders in morphosyntactic, semantic, and pragmatic aspects conditioned by their cognitive level.

All 20 patients were receiving pharmacological treatment at baseline. Seven patients (35 %) received one drug, five patients (25 %) two drugs, two patients (10 %) three drugs, two patients (10 %) four drugs, and four patients (20 %) five drugs. The drugs were used in monotherapy or in combination to improve behavior, but, according to parental reports, did not improve behavior in 11 patients (55 %), partially improved behavior in four (20 %), slightly improved behavior in four others (20 %), or very slightly improved behavior in one (5 %). Drugs received by patients at protocol initiation and associated with cannabis were risperidone in 13, aripiprazole in 10, levomepromazine in six, topiramate in six, melatonin in four, olanzapine in three, valproic acid in two, metformin in two, and sertraline, thioridazine, clonazepam, atomoxetine, and diphenhydramine in one each. Number of drugs received by patients before and during treatment was one in seven patients (35 %), two in five (25 %), three in two (10 %), four in two (10 %), and five in four patients (20 %). No drug-CBD interactions were observed.

Median initial CBD dose was 138.75 mg (range, 35–400) and the median total dose was 363.5 mg (range, 100–700). The mean time of treatment duration was 9.5 months (median, 11 months; range, 6 months–12 months).

The demographic features of our patients are shown in Table 1.

3.2. Efficacy

Five symptoms were selected by each of the 20 families as being the most severe, resulting in 91 severe symptoms/behaviors. Most symptoms were related to aggressive behaviors (self/hetero aggressive), irritability, impulsivity, hyperactivity, repetitive behaviors, exaggerated responses to sensory stimuli and difficulties related to sleeping, eating, and staying dressed. The median number of episodes per week of the

Table 1
Demographic data of our cohort of patients.

General data	Patients (n = 20)	Patients with improvement in at least one severe symptom (n = 18)	Patients with improvement in all severe symptoms (n = 15)
Sex: m (%)	17 (85 %)	15 (83.3)	12 (80 %)
Mean age years (±SD)	10 (±4.58)	10.66 (±4.11)	11.13 (±4.08)
Median weight kg (IQR)	45 (32–71)	45 (34–66.5)	45 (33–74)
Mean final CBD dose (±SD) mg	329.73 (±204.26)	375 (±188.4)	408 (±188.4)
Number of drugs used (±SD)	4.5 (±2.29)	4.2 (±2.18)	4.3 (±2.3)
Number of drugs with dose lowering (±SD)	1.75 (±0.95)	1.75 (±0.95)	1.75 (±0.95)
Mean number of discontinued drugs (±SD)	2 (±1.15)	2 (±1.15)	2 (±1.15)
Median treatment duration days (IQR)	306 (14–368)	317 (185–368)	325 (185–368)

nine most severe symptoms/behavior categories selected by the parents before CBD initiation and after 3 months on the treatment is shown in Table 2. Of the symptoms reported, improvement in at least one was observed after initiation of CBD treatment in 18 patients (90 %) and no improvement in two (10 %) (1 worsening, 1 non-response). Overall, 83.5 % (n = 76) of all symptoms reported improved. Of those that improved, 50 % (n = 38) were much improved (>75 %), while 40.7 % (n = 31) were improved (50–74 %) and 9.3 % (n = 7) were slightly improved (25–49 %) (Fig. 1).

The severity of restricted and repetitive behavior was measured using the RBS-R. At baseline, severity was mild in one (5.5 %), moderate in 11 (61.1 %), and severe in six patients (33.3 %). After CBD initiation, severity was mild in three patients (16.6 %), moderate in 13 (72.2 %), and severe in the remaining two patients (11.1 %). Therefore, severity improved in nine (50 %), remained unchanged in seven (38.8 %), and worsened in two patients (11.1 %).

In the Maladaptive Behavior Domain of the VABS-II, the patients had a median score of 22 (clinically significant) at baseline, which decreased to a median score of 20.5 (elevated) after CBD treatment.

On the ABC, at baseline patients had a median score of 28.7 (range, 16–45) on irritability, of 10 (range, 2–29) on social withdrawal, and of 30 (range, 9–36) on hyperactivity, compared to 19.8 (range, 9–36), 6 (range, 0–14), and 21 (range, 9–36), respectively, after CBD initiation. The mean improvement in irritability was 33 %. The irritability score decreased in 15 patients, with a reduction between 1 % and 24 % in three, between 24 % and 49 % in 10, between 50 % and 74 % in one, and >75 % in the remaining patient. Regarding social withdrawal, a mean improvement of 40 % was found. The social withdrawal score decreased in 15 patients, with a reduction between 1 % and 24 % in four, between 24 % and 49 % in six, between 50 % and 74 % in two, and >75 % in three patients. The hyperactivity showed a mean improvement of 30 % and the score decreased in 16 patients, of whom eight had a reduction between 1 % and 24 %, two between 24 % and 49 %, five between 50 % and 74 %, and the remaining patient of >75 %.

The Pediatric Sleep CGI-S showed that all 18 patients who remained on the treatment had sleep difficulties at baseline without significant differences at three months on CBD. In all patients at least one item of the scale continued to be affected but, if evaluated individually, we found that difficulties to fall asleep and remain sleeping independently apart from parents were observed in 13 patients (72.2 %) at baseline, who all continued with the same difficulties, although they were less severe in the majority of them. Bedtime resistance was seen in 16 patients (88.8 %) before and nine patients (50 %) after CBD initiation, delayed sleep onset in 16 (88.8 %) versus seven patients (38.8 %), and nocturnal awakenings in 14 (77.7 %) versus seven patients (38.8 %).

The median score on the AFEQ was 154.5 at baseline and decreased to 144.5 after starting CBD, while the median score on the PSS at baseline was 53 versus 49 after CBD initiation, which was not statistically significant.

The results of the behavioral assessments before CBD initiation and at 3 months after CBD initiation in our series of patients with ASD are shown in Table 3.

Table 2
Number of episodes per week of the 9 most severe symptoms/behavior categories selected by the parents before CBD initiation and after 3 months on the treatment.

Symptom/behavior (n = number of patients)	Before CBD initiation	After CBD initiation (assessment at 3 months post-CBD)	p value[a]
Aggressive behavior (n = 15) Median (IQR)	70 (21–140)	12 (3–30)	0.001
Irritability (n = 13) Median (IQR)	35 (21–70)	10 (3–14)	0.001
Hyperactivity (n = 10) Median (IQR)	38 (35–42)	12 (9–25)	0.005
Repetitive behavior (n = 10) Median (IQR)	95 (40–210)	39 (25–100)	0.005
Sleep difficulties (n = 5) Median (IQR)	7 (5–7)	0 (0–1)	0.042
Impulsivity (n = 4) Median (IQR)	39 (32–49)	13 (8–15)	0.068
Difficulties staying dressed (n = 4) Median (IQR)	25 (13–67)	3 (2–5)	0.06
Exaggerated response to stimuli (n = 2) Median (IQR)	25 (14–35)	2 (0–3)	0.18
Eating difficulties (n = 2) Median (IQR)	28 (28–28)[c]	7 (3–10)[b]	0.18

[a] IQR, interquartile range.
[b] Wilcoxon Signed-Rank Test.
[c] Episodes were 28 in both patients.

3.3. Safety

Adverse effects were reported in 13 patients (65 %), mainly consisting of increased irritability and decreased appetite. All the adverse effects were mild or moderate and transient. The adverse effects are detailed in Table 4. They coincided with a dose increase or a febrile episode and were resolved by reverting to the previous dose or following the remission of the infectious disease. After decreasing the dose, slow uptitration was carefully tried again.

3.4. Follow-up

The patients were evaluated every three months. During the study period, independently of the time of follow-up, the patients kept a similar performance on all the scales. Due to the good response in the majority of patients, a relatively low dose was used and not further increased, resulting in improvement of the quality of life of the patients and their families.

Throughout the follow-up period, dosage adjustments or discontinuation of different medications was achieved in eight patients (40 %). The supervision of these reductions varied; some were guided by the principal investigator, while others resulted from parental decisions, because of symptom improvement during CBD treatment. Importantly, no excessive weight gain was observed in any of the patients throughout the protocol.

In two patients, CBD was discontinued, due to increased irritability in one and because of non-compliance in the other.

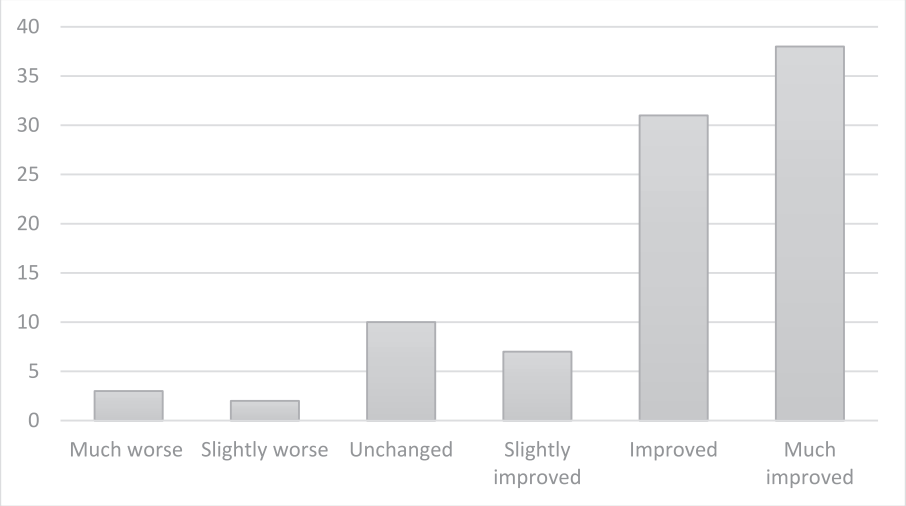

Fig. 1. Evaluation of the symptoms selected by the parents ($n = 91$).

Table 3
Behavioral assessments before CBD initiation and at 3 months after CBD initiation in our series of patients with autism spectrum disorders.

Behavioral assessments	Before CBD initiation n = 20	After CBD initiation (assessment at 3 months post-CBD) n = 18
RBS-R	Mild 1, moderate 11, severe 6	Mild 3, moderate 13, severe 2
Maladaptive Behavior Domain VABS-II – score median (range)	22 (19–26)	20.5 (19–24)
ABC score median (range)		
	28.7 (16–45)	19.8 (9–36)
- Irritability	10 (2–29)	6 (0–14)
- Social withdrawal	30 (9–36)	21 (9–36)
- Hyperactivity		
AFEQ – score median (range)	154.5 (128–198)	144.5 (123–169)
PSS – score median (range)	53 (38–70)	49 (29–68)
Pediatric Sleep CGI-S – number of patients (%)		
	13 patients (72.2 %)	13 patients (72.2 %)
- Difficulty falling asleep and sleeping independently	16 patients (88.8 %)	9 patients (50 %)
- Bedtime resistance	16 patients (88.8 %)	7 patients (38.8 %)
- Delayed sleep onset	14 patients (77.7 %)	7 patients (38.8 %)
- Nocturnal awakenings		

Abbreviations: ABC, Aberrant Behavior Checklist; AFEQ, Autism Family Experience Questionnaire; CGI—S, Clinical Global Impressions Scale severity; PSS, Parental stress scale; RBS-R, Repetitive Behaviors Scale-Revised; VABS-II, Vineland Adaptive Behavior Scales-II.

Table 4
Adverse effects observed in our series of patients.

Adverse effect	Number of patients
Increase in irritability	6
Decreased appetite	2
Increase in impulsivity	1
Hetero-aggressivity	1
Hand tremor	1
Worsening behavior	1
Fever	1
Sleep disturbances	1
Nervousness	1
Increase in anxiety	1
Increase in auto-aggressivity	1

4. Discussion

Our study was driven by the need to provide support to families encountering severe behavioral challenges leading to significant dysfunction and family stress. We evaluated CBD for the treatment of 20 children aged between 3 and 17 years, diagnosed with ASD Level 2 or 3 and associated ID and language impairment. All children exhibited behaviors classified by their families as "severely disruptive," affecting the quality of life of the children and their families. These behaviors persisted despite the use of multiple medications, either as monotherapy or in combination. Additionally, most patients experienced clear adverse effects typical of these drugs, such as a significant increase in body weight.

CBD has emerged as an interesting option for the treatment of ASD with a good safety profile (Stolar et al., 2022). ASD is currently the second-largest condition for which the drug is used, surpassed only by epilepsy. Over recent years, different studies using CBD for children with ASD have been published using different evaluation tools (Aran et al., 2021; Barchel et al., 2019; Fleury-Teixeira et al., 2019; Bar-Lev Schleider et al., 2019; Silva et al., 2024; Bilge and Ekici, 2021; Hacohen et al., 2022). Interestingly, Aran et al. (2021) compared three groups of patients in a cross-over study using whole-plant CBD, purified CBD, and placebo.

Autism is a highly heterogeneous disorder characterized by various core and non-core symptoms, along with a range of comorbidities. Questionnaires often fail to capture the diverse symptomatology of individual patients, particularly those with more severe manifestations (Aran et al., 2021). Therefore, as the primary objective of our study, families were asked to identify five of the most disruptive symptoms/behaviors exhibited by their children. The effectiveness of CBD was then assessed based on the reduction or improvement of these identified behaviors following CBD initiation. Improvement in at least one symptom was observed after CBD initiation in 18 patients (90 %), while no improvement was noted in two patients (10 %). Effectiveness was further assessed using various scales. In correlation to the improvement of the primary objective, the score in the Maladaptive Behavior Domain of the VABS-II responded by the parents decreased.

Severity of irritability, social withdrawal, and hyperactivity improved at least 30 % in the cohort, which is similar to findings in other studies (Barchel et al., 2019; Fleury-Teixeira et al., 2019; Hacohen et al., 2022; Aran et al., 2021). On the other hand, the severity of restricted and repetitive behavior, which could be measured in 18, improved in nine (50 %), remained unchanged in seven (38.8 %), and worsened in two patients (11.1 %). Hacohen et al. (2022), who also studied this item,

found that a significant improvement in symptoms of restricted and repetitive behavior was apparent only in parent reports evaluated through the Restricted and Repetitive Behavior domain of the Social responsiveness scale, 2nd edition (SRS-2) but not in clinical reports of the same subscale on the Autism diagnostic observation schedule, 2nd edition (ADOS-2).

Regarding sleep, in our series only a slight improvement in sleep patterns was found, similar to Bar-Lev Schleider et al. (2019), while other authors found more significant improvements (Silva et al., 2024; Barchel et al., 2019; Fleury-Teixeira et al., 2019). Nevertheless, in a randomized trial specifically evaluating sleep in children with ASD, comparing whole-plant cannabis extract (CBD-THC ratio 20:1), purified CBD, and oral placebo, no sleep improvements were found (Schnapp et al., 2022).

In previous studies, the most common adverse effects were somnolence, decreased appetite, irritability, and weight loss, which were usually mild or moderate and generally not a reason to discontinue treatment (Barchel et al., 2019; Aran et al., 2021; Fleury-Teixeira et al., 2019; Bar-Lev Schleider et al., 2019; Silva et al., 2024). Adverse effects were similar in our study, although remarkably, somnolence, as reported by other studies (Bar-Lev Schleider et al., 2019; Aran et al., 2019b), was not observed in any of our patients. In our study, one patient stopped the treatment due to increased irritability. In a study by Aran et al. (2019b) a severe adverse effect was observed in an adolescent girl who had a psychotic event. In the study by Hacohen et al. (2022), 12/82 discontinued, due to increased aggression or anxiety, weight gain, abdominal pain, increased hyperactivity, and a decrease in communication. On the other hand, as children with ASD generally take concomitant medications, it is difficult to determine whether these adverse events were caused by the CBD or a negative synergy between CBD and other drugs. Fleury-Teixeira et al. (2019) reported that three patients stopped CBD, due to worsening autistic behaviors in two patients, and due to insomnia, irritability, increased heart rate, and worsening of psycho-behavioral crises in the remaining patient. The authors speculated that the former situation may have been due to the unsupervised and sudden cessation of the antipsychotics, while the latter may have been caused by the interaction of CBD with previously prescribed antipsychotic drugs.

An important secondary advantage of CBD is that in a considerable number of cases the number and dosage of other medications can be reduced (Fusar-Poli et al., 2020). In our study, concomitant medications could be decreased or partially stopped in 40 %, but not completely discontinued in any of the patients. In a retrospective study on the use of CBD in 60 children with ASD and severe behavioral problems (Aran et al., 2019a), 33 % received fewer medications or a lower dosage, while 24 % stopped taking medications. In the observational studies by Fleury-Teixeira et al. (2019) and Bar-Lev Schleider et al. (2019), concomitant medication could be completely withdrawn in 20 %, and partially withdrawn or reduced in dosage in 40 % and 5.4 %, respectively.

Quantifying a meaningful qualitative change for the families through a decrease in scores on scales and questionnaires proves challenging, given the subjective nature of parental responses. Moreover, there is a lack of questionnaires and scales that are entirely tailored to objectively measure the diverse comorbidities present in our patient population. In addition, it has been observed that many items in the questionnaires may be inapplicable for some low-functioning participants, leading to invalid scores (Aran et al., 2021).

Unlike most of the previous reports that used CBD-enriched products (Barchel et al., 2019; Fleury-Teixeira et al., 2019; Silva et al., 2024), in our study we used a purified CBD formulation, which was pharmacologically the most reliable among our options. Nevertheless, in the other studies, the ratios of CBD:THC were highly variable, ranging from 9:1 (Silva et al., 2024) to 75:1 (Fleury-Teixeira et al., 2019), making comparisons difficult. In addition, the so-called entourage effect, i.e., the synergistic pharmacological effect of other biochemical compounds of the cannabis plant, may be positive or negative. Aran et al. (2021) found no clear advantages for the whole-plant extract over pure cannabinoids, suggesting that attempts to search for the optimal 'entourage' effect across cannabis strains with the same CBD:THC ratio are likely to be challenging.

Prior research has demonstrated that CBD can inhibit the metabolism of many commonly prescribed medications via Cytochrome P450 (CYP) pathways, potentially increasing the risk of adverse effects or diminishing therapeutic efficacy (Campos et al., 2024). In a retrospective chart review of adverse event reports involving interactions between cannabinoids and psychotropic drugs, Chrobak et al. (2024) found that cannabinoids were implicated in 8 % (20/256) of adverse events associated with the concomitant use of psychotropic drugs and other preparations. Of these, 10 % (2/20) were related to the simultaneous use of sertraline and CBD, while 18 cases (90 %) involved potentially severe adverse effects linked to the combination of THC and CBD. Notably, no side effects were observed in our patient who concomitantly received CBD and sertraline.

Another pharmacokinetic mechanism underlying clinically significant drug interactions involves ATP-binding cassette (ABC) transporters. P-glycoprotein (P-gp), an ABC transporter located at the blood-brain barrier, limits the brain accumulation of central nervous system substrates, including several antipsychotics. P-gp at the blood-brain barrier binds antipsychotic drugs such as risperidone, paliperidone (9-hydroxy risperidone), olanzapine, quetiapine, amisulpride, and aripiprazole, transporting them out of brain tissue and back into the bloodstream. THC exposure has been shown to increase P-gp expression in brain regions critical for risperidone's antipsychotic effects (Brzozowska et al., 2017). Despite risperidone and aripiprazole being the most commonly used antipsychotics in our study, no diminished effect was observed, likely due to the use of a purified CBD product containing minimal THC.

Results on the scales measuring parental stress (Berry and Jones, 1995) and family experience (Leadbitter et al., 2018) did not show significant differences. However, 18/20 families observed improvements in behavior, sleep patterns, and social interaction. The parents reported that CBD positively impacted daily child management, family dynamics, perception of the condition's severity, stress levels, and therefore overall quality of life. These changes were also observed in areas beyond the family setting, including medical, recreational, and therapeutic contexts. Remarkably, in 40 % of the children, medication could be reduced or partially discontinued.

Parental expectations play a significant role in shaping subjective reports of treatment outcomes in children with ASD (Barchel et al., 2019). High expectations from a treatment, such as cannabinoid therapy, may lead parents to perceive improvements due to a placebo effect, as evidenced by previous studies highlighting moderate placebo responses in ASD trials. Interestingly, clinician ratings have been associated with a stronger placebo effect than those of parents. While parental optimism often correlates with increased placebo responses, studies also indicate that caregiver strain and reduced hopefulness at the start of a trial may lower this effect (Masi et al., 2017). Importantly, parents remain a vital source of information for assessing both the child's progress and adverse events, especially in populations where communication limitations necessitate caregiver reporting (Barchel et al., 2019).

In a recent study by Aran et al. (2023) evaluating child and parent characteristics associated with the placebo response to CBD treatment in a large cohort of children and adolescents with ASD, contrary to what was expected, parental expectations regarding cannabinoid treatment were not associated with the placebo response.

Our study has several limitations. First, the small sample size limits the generalizability of the findings. Additionally, the open-label study design may have introduced positive bias among parents and physicians due to the placebo effect. The absence of a control group, a comparison with whole-plant extracts (entourage effect), and a placebo group further restricts the ability to draw definitive conclusions about the intervention's effectiveness. Moreover, the scales and questionnaires used

were not specifically tailored to the unique circumstances of individual patients, and the subjective nature of the responses complicates data interpretation. The content of these tools may also fail to comprehensively capture the full impact of the intervention. Consequently, the findings must be interpreted with caution due to these study design limitations. Further research is needed to generate more robust data on the efficacy, tolerability, and safety of CBD for managing ASD-associated symptoms and comorbidities.

5. Conclusions

Our findings suggest that treatment with purified CBD is effective and safe, potentially benefiting patients with severe ASD by improving core symptoms, including repetitive behaviors and social interaction, as well as associated comorbidities such as disruptive behaviors and sleep disorders. Additionally, families reported an improvement in overall quality of life. However, these results should be interpreted in light of the study's limitations, and further research is required to provide more robust data on the efficacy, tolerability, and safety of CBD for managing ASD-associated symptoms and comorbidities.

CRediT authorship contribution statement

Pablo Sebastián Fortini: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Javier J. Toibaro:** Formal analysis. **Roberto H. Caraballo:** Writing – review & editing, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Patient consent statement

Parents of the participants provided written informed consent and written assent was obtained from participants when appropriate.

Permission to reproduce material from other sources

Permission to use the Parental Stress Scale was obtained from Judy O. Berry, EdD, Professor Emerita of Psychology, the University of Tulsa.

Ethics approval statement

The study was approved by the Bioethics in Research Committee (accreditation nr. MSGCBA 00012) before participant enrollment.

Funding statement

Laboratory Alef, Argentina, provided the CBD for the families as well as support for the methodology and statistical analysis.

Declaration of competing interest

The authors have no conflict of interests to declare.

Acknowledgements

We would like to thank the parents of the children with ASD who participated in this study and generously shared their observations, Mariana Fazzina for her assistance in the methodology.

Data availability

Data will be made available on request.

References

Adler, B.A., Wink, L.K., Early, M., Shaffer, R., Minshawi, N., McDougle, C.J., et al., 2015. Drug-refractory aggression, self-injurious behavior, and severe tantrums in autism spectrum disorders: a chart review study. Autism 19 (1), 102–106. https://doi.org/10.1177/1362361314524641.

Agarwal, R., Burke, S.L., Maddux, M., 2019. Current state of evidence of cannabis utilization for treatment of autism spectrum disorders. BMC Psychiatry 29;19(1): 328. https://doi.org/10.1186/s12888-019-2259-4.

Aman, M.G., Singh, N.N., Stewart, A.W., Field, C.J., 1985. The aberrant behavior checklist: a behavior rating scale for the assessment of treatment effects. Am. J. Ment. Defic. 89 (5), 485–491.

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders, 5th ed. American Psychiatric Association, Arlington, VA.

Anagnostou, E., 2018. Clinical trials in autism spectrum disorder: evidence, challenges and future directions. Curr. Opin. Neurol. 31, 119–125. https://doi.org/10.1097/WCO.0000000000000542.

Aran, A., Cassuto, H., Lubotzky, A., Wattad, N., Hazan, E., 2019a. Brief report: cannabidiol-rich cannabis in children with autism spectrum disorder and severe behavioral problems-a retrospective feasibility study. J. Autism Dev. Disord. 49 (3), 1284–1288. https://doi.org/10.1007/s10803-018-3808-2.

Aran, A., Eylon, M., Harel, M., Polianski, L., Nemirovski, A., Tepper, S., Schnapp, A., Cassuto, H., Wattad, N., Tam, J., 2019b. Lower circulating endocannabinoid levels in children with autism spectrum disorder. Mol. Autism. 10, 2. https://doi.org/10.1186/s13229-019-0256-6.

Aran, A., Harel, M., Cassuto, H., Polyansky, L., Schnapp, A., Wattad, N., Shmueli, D., Golan, D., Castellanos, F.X., 2021. Cannabinoid treatment for autism: a proof-of-concept randomized trial. Mol. Autism. 12 (1), 6. https://doi.org/10.1186/s13229-021-00420-2.

Aran, A., Harel, M., Ovadia, A., Shalgy, S., Cayam-Rand, D., 2023. Mediators of placebo response to cannabinoid treatment in children with autism spectrum disorder. J. Clin. Med. 12 (9), 3098. https://doi.org/10.3390/jcm12093098.

Barchel, D., Stolar, O., De-Haan, T., Ziv-Baran, T., Saban, N., Fuchs, D.O., Koren, G., Berkovitch, M., 2019. Oral cannabidiol use in children with autism spectrum disorder to treat related symptoms and co-morbidities. Front. Pharmacol. 9, 1521. https://doi.org/10.3389/fphar.2018.01521.

Bar-Lev Schleider, L., Mechoulam, R., Saban, N., Meiri, G., Novack, V., 2019. Real life experience of medical cannabis treatment in autism: analysis of safety and efficacy. Sci. Rep. 9 (1), 200. https://doi.org/10.1038/s41598-018-37570-y.

Bauman, M.L., 2010. Medical comorbidities in autism: challenges to diagnosis and treatment. Neurotherapeutics 7 (3), 320–327. https://doi.org/10.1016/j.nurt.2010.06.001.

Berry, J.O., Jones, W.H., 1995. The parental stress scale: initial psychometric evidence. J. Soc. Pers. Relat. 12, 463–472.

Bilge, S., Ekici, B., 2021. CBD-enriched cannabis for autism spectrum disorder: an experience of a single center in Turkey and reviews of the literature. J Cannabis Res. 16;3(1):53. https://doi.org/10.1186/s42238-021-00108-7.

Bodfish, J.W., Symons, F.J., Parker, D.E., Lewis, M.H., 2000. Varieties of repetitive behavior in autism: comparisons to mental retardation. J. Autism Dev. Disord. 30 (3), 237–243. https://doi.org/10.1023/a:1005596502855.

Brzozowska, N.I., de Tonnerre, E.J., Li, K.M., Wang, X.S., Boucher, A.A., Callaghan, P.D., Kuligowski, M., Wong, A., Arnold, J.C., 2017. The differential binding of antipsychotic drugs to the ABC transporter P-glycoprotein predicts cannabinoid-antipsychotic drug interactions. Neuropsychopharmacology 42 (11), 2222–2231. https://doi.org/10.1038/npp.2017.50.

Campos, M.G., China, M., Cláudio, M., Capinha, M., Torres, R., Oliveira, S., Fortuna, A., 2024. Drug-cannabinoid interactions in selected therapeutics for symptoms associated with epilepsy, autism spectrum disorder, cancer, multiple sclerosis, and pain. Pharmaceuticals 17 (5), 613. https://doi.org/10.3390/ph17050613.

Chakrabarti, B., Persico, A., Battista, N., Maccarrone, M., 2015. Endocannabinoid signaling in autism. Neurotherapeutics 12 (4), 837–847. https://doi.org/10.1007/s13311-015-0371-9.

Chrobak, A.A., Woroń, J., Siwek, M., 2024. Green rush and red warnings: retrospective chart review of adverse events of interactions between cannabinoids and psychotropic drugs. Front. Pharmacol. 15, 1500312. https://doi.org/10.3389/fphar.2024.1500312.

Fleury-Teixeira, P., Caixeta, F.V., Ramires da Silva, L.C., Brasil-Neto, J.P., Malcher-Lopes, R., 2019. Effects of CBD-enriched cannabis sativa extract on autism spectrum disorder symptoms: an observational study of 18 participants undergoing compassionate use. Front. Neurol. 10, 1145. https://doi.org/10.3389/fneur.2019.01145.

Fusar-Poli, L., Cavone, V., Tinacci, S., Concas, I., Petralia, A., Signorelli, M.S., Díaz-Caneja, C.M., Aguglia, E., 2020. Cannabinoids for people with ASD: a systematic review of published and ongoing studies. Brain Sci. 10 (9), 572. https://doi.org/10.3390/brainsci10090572.

Goel, R., Hong, J.S., Findling, R.L., Ji, N.Y., 2018. An update on pharmacotherapy of autism spectrum disorder in children and adolescents. Int. Rev. Psychiatry 30 (1), 78–95. https://doi.org/10.1080/09540261.2018.1458706.

Hacohen, M., Stolar, O.E., Berkovitch, M., Elkana, O., Kohn, E., Hazan, A., Heyman, E., Sobol, Y., Waissengreen, D., Gal, E., Dinstein, I., 2022. Children and adolescents with ASD treated with CBD-rich cannabis exhibit significant improvements particularly in social symptoms: an open label study. Transl. Psychiatry 12 (1), 375. https://doi.org/10.1038/s41398-022-02104-8.

Höfer, J., Hoffmann, F., Bachmann, C., 2017. Use of complementary and alternative medicine in children and adolescents with autism spectrum disorder: a systematic review. Autism 21 (4), 387–402. https://doi.org/10.1177/1362361316646559.

Holdman, R., Vigil, D., Robinson, K., Shah, P., Contreras, A.E., 2022. Safety and efficacy of medical cannabis in autism spectrum disorder compared with commonly used medications. Cannabis Cannabinoid Res. 7 (4), 451–463. https://doi.org/10.1089/can.2020.0154.

Karhson, D.S., Krasinska, K.M., Dallaire, J.A., Libove, R.A., Phillips, J.M., Chien, A.S., Garner, J.P., Hardan, A.Y., Parker, K.J., 2018. Plasma anandamide concentrations are lower in children with autism spectrum disorder. Mol. Autism. 9, 18. https://doi.org/10.1186/s13229-018-0203-y.

Leadbitter, K., Aldred, C., McConachie, H., Le Couteur, A., Kapadia, D., Charman, T., Macdonald, W., Salomone, E., Emsley, R., Green, J., PACT Consortium, 2018. The Autism Family Experience Questionnaire (AFEQ): an ecologically-valid, parent-nominated measure of family experience, quality of life and prioritised outcomes for early intervention. J. Autism Dev. Disord. 48 (4), 1052–1062. https://doi.org/10.1007/s10803-017-3350-7.

Malow, B.A., Connolly, H.V., Weiss, S.K., Halbower, A., Goldman, S., Hyman, S.L., Katz, T., Madduri, N., Shui, A., Macklin, E., Reynolds, A.M., 2016. The pediatric sleep clinical global impressions scale-a new tool to measure pediatric insomnia in autism spectrum disorders. J. Dev. Behav. Pediatr. 37 (5), 370–376. https://doi.org/10.1097/DBP.0000000000000307.

Martínez-González, A.E., Piqueras, J.A., Bodfish, J.W., 2021. Adaptación española de la Escala de Conductas Repetitivas Revisada (Repetitive Behavior Scale-Revised, RBS-R). ABA España. https://doi.org/10.26741/978-84-09-28002-5.

Masi, A., DeMayo, M.M., Glozier, N., Guastella, A.J., 2017. An overview of autism spectrum disorder, heterogeneity and treatment options. Neurosci. Bull. 33 (2), 183–193. https://doi.org/10.1007/s12264-017-0100-y.

Mostafavi, M., Gaitanis, J., 2020. Autism spectrum disorder and medical cannabis: review and clinical experience. Semin. Pediatr. Neurol. 35, 100833. https://doi.org/10.1016/j.spen.2020.100833.

Russo, E.B., 2011. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. Br. J. Pharmacol. 163 (7), 1344–1364. https://doi.org/10.1111/j.1476-5381.2011.01238.x.

Russo, E.B., 2017. Cannabis and epilepsy: an ancient treatment returns to the fore. Epilepsy Behav. 70 (Pt B), 292–297. https://doi.org/10.1016/j.yebeh.2016.09.040.

Schnapp, A., Harel, M., Cayam-Rand, D., Cassuto, H., Polyansky, L., Aran, A., 2022. A placebo-controlled trial of cannabinoid treatment for disruptive behavior in children and adolescents with autism spectrum disorder: effects on sleep parameters as measured by the CSHQ. Biomedicines 10 (7), 1685. https://doi.org/10.3390/biomedicines10071685.

Silva, E.A.D. Junior, Medeiros, W.M.B., Santos, J.P.M.D., Sousa, J.M.M., Costa, F.B.D., Pontes, K.M., Borges, T.C., Espínola, C., Segundo, Neto, Andrade E Silva, A.H., Nunes, E.L.G., Alves, N.T., Rosa, M.D.D., Albuquerque, K.L.G.D., 2024. Evaluation of the efficacy and safety of cannabidiol-rich cannabis extract in children with autism spectrum disorder: randomized, double-blind, and placebo-controlled clinical trial. Trends Psychiatry Psychother. 46, e20210396. https://doi.org/10.47626/2237-6089-2021-0396.

Sparrow, S.S., Cicchetti, D., Balla, D.A., 2005. Vineland adaptive behavior scales, second edition (Vineland-II) [database record]. APA PsycTests. https://doi.org/10.1037/t15164-000.

Stolar, O., Hazan, A., Vissoker, R.E., Kishk, I.A., Barchel, D., Lezinger, M., Dagan, A., Treves, N., Meiri, D., Berkovitch, M., Kohn, E., Heyman, E., 2022. Medical cannabis for the treatment of comorbid symptoms in children with autism spectrum disorder: an interim analysis of biochemical safety. Front. Pharmacol. 13, 977484. https://doi.org/10.3389/fphar.2022.977484.

Wink, L.K., Early, M., Schaefer, T., Pottenger, A., Horn, P., McDougle, C.J., Erickson, C. A., 2014. Body mass index change in autism spectrum disorders: comparison of treatment with risperidone and aripiprazole. J. Child Adolesc. Psychopharmacol. 24 (2), 78–82. https://doi.org/10.1089/cap.2013.0099.

Zamberletti, E., Gabaglio, M., Parolaro, D., 2017. The endocannabinoid system and autism spectrum disorders: insights from animal models. Int. J. Mol. Sci. 18 (9), 1916. https://doi.org/10.3390/ijms18091916.

Zou, M., Liu, Y., Xie, S., Wang, L., Li, D., Li, L., Wang, F., Zhang, Y., Xia, W., Sun, C., Wu, L., 2021. Alterations of the endocannabinoid system and its therapeutic potential in autism spectrum disorder. Open Biol. 11 (2), 200306. https://doi.org/10.1098/rsob.200306.