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Trends

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Evaluation of the efficacy and safety of cannabidiol-rich cannabis extract in children with autism spectrum disorder: randomized, double-blind and controlled placebo clinical trial

Cannabinoids in autistic children: clinical trial

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ABSTRACT

Introduction

Autism Spectrum Disorder is characterized by persistent deficits in social communication, social interaction, and restricted and repetitive patterns of behavior. Some studies have shown that substances derived from *Cannabis sativa* improve the quality of life of autistic children without causing serious adverse effects, thus providing a therapeutic alternative.

Method

This was a randomized, double-blind, placebo-controlled clinical trial to evaluate the efficacy and safety of a cannabis extract rich in cannabidiol (CBD) in autistic children. Sixty children, aged between 5 and 11 years, were selected and divided into two groups: the treatment group, which received the CBD-rich cannabis extract, and the control group, which received the placebo, both used the product for a period of 12 weeks. Statistical analysis was done by two-factor mixed analysis of variance (ANOVA two way).

Results

Significant results were found for social interaction [F(1,116)=14.13, p=0.0002)], anxiety [F(1,116)=5.99, p=0.016], psychomotor agitation [F(1,116)=9.22, p=0.003)], number of meals a day [F(1,116)=4.11, p=0.04)] and concentration [F (1,48)=6.75, p=0.01], the latter being significant only in mild autism spectrum disorder. Regarding safety, it was found that only three children in the treatment group (9.7%) had adverse effects, namely dizziness, insomnia, colic and weight gain.

Conclusion

CBD-rich cannabis extract was found to improve one of the diagnostic criteria for ASD (social interaction), as well as often co-existing features, and to have few serious adverse effects.

Keywords: Autistic Spectrum Disorder; child behavior; clinical trial; cannabis; cannabidiol.

INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), characterized by persistent deficits in social communication and social interaction, in multiple contexts, and the presence of restricted and repetitive patterns of behavior, interests, or activities. The DSM-5 also adopts, as specifying criteria, levels of ASD severity, which vary according to the need for support: mild (needs support), moderate (needs substantial support), and severe (needs very substantial support)[1].

There are no treatments proven to target the core features of ASD. The treatments are just symptomatic, aiming mainly to reduce aggressivity and psychomotor agitation symptoms and usually using psychotropic medications to improve these behavioral changes. In order to help patients affected by this disorder, there is a great interest in discovering new therapeutic alternatives to overcome the ineffectiveness of some conventional psychotropic drugs used in ASD's treatment or even suspend them, reducing the adverse effects associated with these drugs[2].

Endocannabinoids, substances that are part of the Endocannabinoid System (ECS), are key modulators of socioemotional responses, cognition, seizure susceptibility, nociception, and neuronal plasticity, and all of these responses are altered in autism[3,4]. Phytocannabinoids, mainly cannabidiol (CBD) and tetrahydrocannabinol (THC), present in several subspecies of the Cannabis genus, have been widely studied as a potential therapeutic alternative for the treatment of symptoms associated with ASD, as they activate the cannabinoid receptors present in the central nervous system, alleviating some symptoms associated with autism[5].

Even though cannabis is becoming a topic of great interest among scientists, it is still difficult to find clinical trials with humans due to the restrictive and legal aspects surrounding the plant[6]. In addition, studies using cannabis for the treatment of difficult-to-control epilepsy have found that in addition to seizure reduction, there was improvement in behavior and social interaction in children who had ASD as a comorbidity[7].

In light of the above, the purpose of this research is to evaluate the efficacy of CBD-rich cannabis extract in children with ASD, monitoring aspects belonging to the DSM-5 diagnostic criteria (social interaction, speech and stereotypes) and other often coexisting ones (aggressiveness, psychomotor agitation, impaired concentration, eating disorders, sleep disorders and anxiety), as well as to assess the tolerability and safety of the therapeutic adjuvant.

METHOD

This research was a randomized, double-blind, placebo-controlled, 12-week clinical trial, following the CONSORT Recommendation. Initially, G Power software was used

to calculate the sample size based *Handen B.L et. al* studies in autistic children who took Donepezil[8]. In it, an alpha of 0.05 and a power of 0.80 were considered. The calculation showed that 62 subjects would be needed (31 per group).

This study included children aged between 5 and 11 years, who lived in the State of Paraíba, Brazil, and in neighboring states (Pernambuco and Rio Grande do Norte), who were diagnosed with ASD through a medical certificate, regardless of having a mild, moderate or severe level of impairment of ASD and whose caregivers signed the Informed Consent Form. This age group was selected because children aged between 5 and 11 years exhibit greater similarity in brain development, making the group more homogeneous during the analysis of the results. In addition, children older than 5 years would be more likely to have developed verbal language and be better able to respond on neuropsychological testing. For these reasons, we chose this age group to participate in this study. Children who had comorbidities such as diabetes mellitus, hypertension, autoimmune diseases, refractory epilepsy and who had used a cannabis product in the last two months before starting the study were excluded.

A wide dissemination was carried out in autism support institutions, with informative lectures and posts in WhatsApp and social media (Facebook and Instagram) to recruit the sample. Thereafter, those interested registered on a website created exclusively for this Clinical Trial, and filled out the sociodemographic questionnaire and the screening scale for autism Childhood Autism Rating Scale (CARS)[9]. The cut-off score is 15 points and CARS was used to assess severity. After applying the eligibility criteria, the researchers contacted the caregivers to provide further clarification about the research.

After recruitment, randomization of the 64 selected children was performed, along with stratification by severity. The randomization was done using the True Random Number Service, available at: www.random.org. All the researchers who had direct contact with the patients were blinded to the treatment provided, except the pharmacy student, who was assigned with delivering the vials, weekly, to the researching physician. Finally, baseline evaluations, performed by the same child and adolescent psychiatrist responsible for this reseach, were scheduled with all children participating in this study, to whom the products for the test were also delivered. The caregiver was instructed to always start with a dose of three drops every 12 hours, preferably in a fasting period and with an interval of at least one hour before or after the use of psychotropic medication, especially antipsychotics, according to the protocol suggested by the product supplier, as it was a plant extract. The starting dose of cannabidiol-rich cannabis extract used in this study was 6 drops daily, increased by 2 drops daily twice a week if necessary and the maximum dose used was 70 drops daily.

This clinical study was conducted on the premises of the Lauro Wanderley da Universidade Federal da Paraíba University Hospital (HULW-UFPB), in the outpatient sector, which is located in João Pessoa, Paraíba, Brazil. This research was approved by the Research Ethics Committee of the Health Sciences Center of the UFPB, under the number CAAE 89392518.4.0000.5188. It was registered in the Brazilian Registry of Clinical Trials (ReBEC) under number 10743.

The product used was CBD-rich cannabis extract at a concentration of 0.5% (5mg/mL), in the ratio of 9CBD:1THC, supplied by the *Associação Brasileira de Apoio Cannabis Esperança* (ABRACE). The extract used throughout the clinical trial was from the same batch, in order to ensure the same phytochemical and pharmacobotanical characteristics during the production of the extract. The CBD-rich cannabis extract and

the product without it had the same consistency, color, odor, and other organoleptic characteristics, making it impossible for patients and the multidisciplinary team accompanying them to discern between the two.

To evaluate the effectiveness of the treatment, we used a semi-structured interview prepared by the authors containing questions related to ASD symptoms and the Autism Treatment Evaluation Checklist (ATEC)[10], which were administered to and answered by caregivers before and after the clinical trial. The number of daily meals was reported by the child's caregiver during the psychiatric consultation after they answered the semi-structured interview questionnaire. In order to assess safety, before starting the study, all children underwent a laboratory evaluation, including renal and liver function tests, as well as complete blood count and fasting glucose levels.

All analyses were performed using R version 4.0.2, a free software available at https://www.r-project.org/. The significance level adopted for all analysis was 5%. Statistical analysis was performed using the mixed variance test for two factors (ANOVA two way). In cases where the null hypothesis was rejected, Tukey's multiple comparisons post-hoc test was applied to verify which groups had significant differences. As there is no non-parametric technique available in the literature regarding mixed analysis of variance for two factors, a simple non-parametric analysis was used for each factor individually, in order to support the results obtained by the parametric technique. The cannabis and control groups, as well as the before and after moments were compared using the Wilcoxon test for independent and dependent samples, respectively.

RESULTS

Sociodemographic analysis of the parents

Regarding parents, different sociodemographic variables were evaluated, among them: age; education; if they had another child, including autism; if father and/or mother worked outside the home or not, and if, because the child was autistic, one of the parents had to stop working, and marital status of parents (Table 1).

Table 1:

Sociodemographic data of the parents of children with ASD.

Variable (parents)	Cannabis group (n = 31)	Control group (n = 29)	Total (n = 60)
Mother age (in conception)	29,00 (29,00) ± 6,09	30,00 (30,00) ± 6,85	29,46 (30,00) ± 6,42
Father age (in conception)	34,05 (34,00) ± 6,69	32,10 (31,00) ± 5,67	33,10 (33,00) ± 6,21
Mother education			
Incomplete elementary	1 (3,57)	2 (7,69)	3 (5,56)
Complete elementary	0 (0,00)	1 (3,85)	1 (1,85)
Incomplete secondary	2 (7,14)	0 (0,00)	2 (3,70)
Complete secondary	9 (32,14)	7 (26,92)	16 (29,63)
Incomplete higher	4 (14,29)	6 (23,08)	10 (18,52)
Complete higher	6 (21,43)	4 (15,38)	10 (18,52)
Postgraduation	6 (21,43)	6 (23,08)	12 (22,22)
Father education			
Incomplete elementary	0 (0,00)	1 (5,56)	1 (2,63)
Complete elementary	1 (5,00)	1 (5,56)	2 (5,26)
Incomplete secondary	3 (15,00)	6 (33,33)	9 (23,68)
Complete secondary	2 (10,00)	2 (11,11)	4 (10,53)
Incomplete higher	8 (40,00)	4 (22,22)	12 (31,58)
Complete higher	6 (30,00)	4 (22,22)	10 (26,32)
Other children			
No	11 (35,48)	10 (34,48)	21 (35,00)
Yes	14 (45,16)	14 (48,28)	28 (46,67)
yes and autistic	6 (19,35)	5 (17,24)	11 (18,33)
Working parents			
No	1 (3,23)	2 (6,90)	3 (5,00)
Yes	20 (64,52)	14 (48,28)	34 (56,67)
one of the parents had to stop	10 (32,26)	13 (44,83)	23 (38,33)

Father or mother civil status			
Single	8 (25,81)	6 (20,69)	14 (23,33)
Married	15 (48,39)	17 (58,62)	32 (53,33)
Divorced	8 (25,81)	5 (17,24)	13 (21,67)
other	0 (0,00)	1 (3,45)	1 (1,67)

Qualitative variables: n (%)
Quantitative variables: average (median) ± standard deviation.

Sociodemographic analysis of the children

In general, there are not significant differences between the treatment groups for the sociodemographic variables evaluated in the children with ASD who participated in this study. However, it is important to observe if the child was undergoing any professional intervention healthcare for ASD (public, private, or mixed) and the corresponding professional(s) (occupational therapist, physical therapist, speech therapist, psychologist, psychopedagogue, or others); whether the child was using psychotropic drugs before, during, and after the clinical trial; whether or not the child had food selectivity and if this eating pattern was modified; the severity of ASD (mild: needs support, moderate: needs substantial support, and severe: needs very substantial support, according to DSM-5 classification); whether there were adverse effects with the use of the product (CBD-rich extract or placebo); amount of drops of the product at the end, since the increase was gradual and as directed by the researcher; whether the caregiver was in doubt, did not notice or could not see improvement with the test product, at the final consultation, before researcher and participants knew if the child was in the treated or placebo group. And because the coronavirus pandemic (COVID-19) outbreak had occurred during the clinical trial, the children were isolated at home or could not receive professional attention, causing changes to their routine, which in itself is a disorganizing factor for those with ASD, so the researchers introduced, in the final evaluation, the parents' report on the interference of isolation on their child's symptoms (Table 2).

Table 2:

Sociodemographic data and information about the children participating in the research.

Qualitative variables: n (%)

Variable (children)	Cannabis group (n = 31)	Control group (n = 29)	Total (n = 60)
Gender			
Male	25 (80,65)	27 (93,10)	52 (86,67)
Female	6 (19,35)	2 (6,90)	8 (13,33)
Age	7,64 (7,00) ± 1,76	7,72 (7,00) ± 1,75	7,68 (7,00) ± 1,74
Child education			
Does not attend	2 (6,45)	2 (6,90)	4 (6,67)
Beneath the expected grading	4 (12,90)	6 (20,69)	10 (16,67)
Within the expected grading	25 (80,65)	21 (72,41)	46 (76,67)
Treatment type			
Does not	6 (19,35)	7 (24,14)	13 (21,67)
Public	14 (45,16)	9 (31,03)	23 (38,33)
Private	7 (22,58)	11 (37,93)	18 (30,00)
mixed	4 (12,90)	2 (6,90)	6 (10,00)
Occupational therapy	16 (51,61)	17 (58,62)	33 (55,00)
Physiotherapy	1 (3,23)	1 (3,45)	2 (3,33)
Fonoaudiology	19 (61,29)	20 (68,97)	39 (65,00)
Psychology	15 (48,39)	17 (58,62)	32 (53,33)
Psychopedagogy	11 (35,48)	12 (41,38)	23 (38,33)
Other treatments	6 (19,35)	2 (6,90)	8 (13,33)
Psychotropics usage			
no	17 (54,84)	10 (34,48)	27 (45,00)
yes and continued	12 (38,71)	16 (55,17)	28 (46,67)
Uses and stopped	1 (3,23)	2 (6,90)	3 (5,00)
Did not use and started	1 (3,23)	1 (3,45)	2 (3,33)
Selective eating			
No	16 (51,61)	17 (58,62)	33 (55,00)
yes and continued	8 (25,81)	7 (24,14)	15 (25,00)
Yes and stopped	7 (22,58)	5 (17,24)	12 (20,00)
Severity			
Mild	13 (41,94)	13 (44,83)	26 (43,33)
Moderate	16 (51,61)	13 (44,83)	29 (48,33)
Severe	2 (6,45)	3 (10,34)	5 (8,33)
Adverse side effects	4 (12,90)	5 (17,24)	9 (15,00)
Subjective improvement			
No	7 (22,58)	12 (41,38)	19 (31,67)
Doubt	3 (9,68)	7 (24,14)	10 (16,67)
Yes	21 (67,74)	10 (34,48)	31 (51,67)
Quantity of drops used	47,42 (52,00) ± 15,22	40,96 (44,00) ± 18,86	44,30(50,00)± 17,23
Isolation interference			
no	19 (61,29)	19 (65,52)	38 (63,33)
Only in the beginning	4 (12,90)	1 (3,45)	5 (8,33)
yes	8 (25,81)	9 (31,03)	17 (28,33)

Quantitative variables: average (median) ± standard deviation.

Analysis of the Semi-Structured Interview, ATEC and CARS

Important variables associated with ASD were evaluated through a semi-structured questionnaire. The symptoms evaluated were aggressiveness; psychomotor agitation; concentration; meals (number of meals/day); sleep (number of hours of sleep/day); social interaction with peers; verbal language (speech); anxiety; repetitive and stereotyped movements (stereotypies). The mean scores of the ATEC scales (and its subdivisions: ATEC L, related to language; ATEC S, related to socialization; ATEC P, related to sensory and cognitive perception; ATEC SC, related to health, physical aspects and behavior; and ATEC T, related to the total sum of the scale) and CARS were also calculated (Table 3).

Table 3:
Evaluation of different variables in children with ASD in the Placebo Group and the Cannabis Group. Results are expressed as average (median) ± standard deviation.

Variable	Placebo Group (n = 29)	Cannabis Group (n=31)	Value p
Aggressivity	1,39 (1,00) ± 1,36	0,81 (0,00) ± 1,05	0,2149
Psychomotor agitation	2,65 (3,00) ± 1,14	1,64 (2,00) ± 1,28	0,00295**
Concentration	2,96 (3,00) ± 0,86	1,71 (2,00) ± 1,07	0,269
Meals	0,38 (0,00) ± 0,82	1,32 (0,00) ± 1,90	0,045*
Sleep	0,28 (0,00) ± 0,59	0,77 (0,00) ± 1,61	0,0711
Social Interaction	2,83 (3,00) ± 1,10	1,68 (2,00) ± 1,01	0,000268***
Speech	1,72 (1,00) ± 1,55	1,32 (1,00) ± 1,42	0,3918
Anxiety	2,90 (3,00) ± 1,23	1,84 (2,00) ± 1,39	0,0159*
Stereotypy	2,07 (2,00) ± 1,03	1,45 (1,00) ± 1,06	0,3853
ATEC L	13,14 (13,00) ± 8,18	12,16 (12,00) ± 7,49	0,254
ATEC S	17,83 (18,00) ± 9,83	13,64 (15,00) ± 6,31	0,113
ATEC P	16,86 (18,00) ± 8,53	13,68 (13,00) ± 7,77	0,212

ATEC SC	27,17 (25,00) ± 11,03	25,35 (25,00) ± 10,79	0,119
ATEC T	75,00 (78,00) ± 32,89	64,84 (63,00) ± 26,82	0,098
CARS	37,83 (39,00) ± 9,02	33,47 (31,00) ± 8,48	0,188

All p-values were calculated between the cannabis after versus placebo after groups using the ANOVA TWO WAY test followed by TUKEY and WILCOXON
*p<0,05; **p<0,01; ***p<0,001

It is possible to observe, according to the semi-structured interview, that children who received the CBD-rich cannabis extract showed a significant improvement in psychomotor agitation, started to accept more meals a day, much improved social interaction and were less anxious, when compared to children in the placebo group, suggesting improvement in some symptoms associated with the ASD condition. On the other hand, regarding the 'concentration' variable, it was possible to observe that only mild autistic children who received the CBD-rich cannabis extract showed significant improvement in this variable (Table 4). For this reason, it is possible to suggest that there is a difference between the severity of the ASD for only 'concentration' evaluated variable.

Table 4:
Mixed analysis of variance for two factors (R software version 4.0.2).

Variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Social Interaction	1	17.63	17.633	14.133	0.000268***
Residuals	116	144.73	1.248		
Psychomotor Agitation	1	14.70	14.700	9.225	0.00295**
Residuals	116	184.84	1.593		
Anxiety	1	10.21	10.208	5.989	0.0159*

Residuals	116	197.73	1.705		
Number of meals per day	1	9.63	9.633	4.109	0.045*
Residuals	116	271.99	2.345		
Concentration (Mild Group)	1	5.56	5.558	6.747	0.0124*
Residuals	48	39.54	0.824		

All p-values were calculated between the cannabis after versus placebo after groups using the ANOVA TWO WAY test followed by TUKEY and WILCOXON
*p<0,05; **p<0,01; ***p<0,001

During recruitment and laboratory testing prior to the start of the clinical trial, four children dropped out of the study, three were recruited for the control group and one for the cannabis group, thus resulting in a final sample of 60 children (31 in the treatment group and 29 in the placebo group). The reason was that they did not live in the city where the clinical trial was executed and had difficulty travelling there. Furthermore, we also analyzed some important hematological parameters, including complete hemogram, glycemia, aspartate aminotransferase (ALT), alanine aminotransferase (AST), urea and creatinine, and it was possible to observe that all these parameters were within normal limits in all children.

DISCUSSION

Even though the CBD-rich extract was used at a low concentration (2.5mg/mL), using 3 drops twice a day, and the basis dose was determined by the ABRACE protocol, improvements in social interaction, psychomotor agitation, number of meals, anxiety, and concentration were observed, and the adverse effects experienced by a few of the children were mild and transient. Concentration improvement results were only for the mild group.

The sample was composed of 86.7% male children and was not selected by gender, but it is known that ASD is more common in boys than in girls [5,15]. Females, as a rule, are more rarely affected (4 boys to 1 girl for autism and 10 boys to 1 girl, considering Asperger's Syndrome). This pattern led to the hypothesis of a "female protective effect", a purported biological aspect by which females require a greater etiological "burden" to manifest autism[16,17]. Enrollment for possible participation in the clinical trial was open to the general population, but there were significantly fewer female children (296 children enrolled, 45 of which were female, thus corresponding to only 15.2%), and 8 girls (13.3%) and 52 boys (86.7%) were selected for the clinical trial.

Concerning the challenges of the COVID-19 pandemic for autistic people, given the importance of routine in their lives, families with autistic children face enormous challenges to mitigate the impact of the condition, as they often fail to carry out preventive measures. Sudden changes, such as social isolation, can cause emotional and behavioral changes, such as anxiety, agitation and agressivity[18]. It is not clear whether COVID-19 had any impact on the research, but differences were observed in medical consultations. For this reason, parents were asked, at the end, if the social isolation, which led to the temporary discontinuation of multidisciplinary treatments and consequent changes in routine, had interfered with the children's functioning and 71.7% reported that there was no interference with any significant impairment.

The neuropsychological assessment is crucial to complement the ASD's diagnosis and to observe the child's evolution during the use of some medication intervention. It was also important that Neuropsychological tests, such as executive functions, Theory of Mind, empathy and attention[19], were performed to evaluate the main psychological functions related to ASD in several areas, such as severity of autism and verbal language.

Efficacy of CBD-rich cannabis extract

One of the core symptoms, which is described as one of the diagnostic criteria, in DSM-5, is persistent impairment in social interaction, which this research brought, as a result, the most robust improvement ($p<0.001$). The reduction of psychomotor agitation was of great relevance. ASD children's parents often reported several food restriction problems and inadequate diet by their children [20,21]. These issues were observed in our sample, in which the caregivers said that many participants had fewer daily meals than desired, had difficulty eating due to sensory and food selectivity issues. An improvement was found in eating habits with the use of cannabis, in the parameter "meals". Anxiety, which accompanies many children with ASD, can lead to behavioral changes, given that they often cannot express what they feel, also leading to psychological suffering[22,23.24].

A major complaint of parents refers to concentration, so it was very important to analyze it according to the severities of ASD, since mild children possibly have less cognitive impairment, since they require less support, which could suggest an improvement in the ability to concentrate only in this severity, as found in this research, but we cannot confirm, through this clinical trial, such a hypothesis.

In a study of 400 individuals in New Zealand evaluating the prescription of CBD in clinical practice also assessed neurological symptoms, which included Parkinson's disease, multiple sclerosis, epilepsy, autism spectrum disorder with challenging behavior, amyotrophic lateral sclerosis, multiple system atrophy, chronic pain, various neuropathies, and tremors. Mental health symptoms include anxiety disorders, depressive disorders, post-traumatic stress disorder, stress disorder, and insomnia[25].

However, so far, there is no randomized, double-blind, placebo-controlled clinical trial, besides stratification by severity, with a sample composed only of children with ASD.

A research study with a sample of 60 individuals, with the average being equal to 11 years old, but was retrospective, showed improvements in behavioral outbursts (61%) and anxiety (39%)[26]. A prospective study with cannabis use, which also included adults (53 participants aged 4 to 22 years), and with biweekly evaluations through structured interviews, resulted in 67.6% improvement in self-injury and bouts of anger, 68.4% improvement in hyperactivity, 71.4% improvement in sleep disturbances and 47.1% improvement in anxiety, and mild to moderate adverse effects, such as drowsiness and decreased appetite[27].

In another prospective study, 188 children were observed for six months, with all subjects receiving cannabis. From the results of structured questionnaires filled out by their parents, it was found that 30.1% of the subjects presented significant improvement, 53.7% moderate improvement, 6.4% slight improvement and 8.6% presented no improvement and agitation (6.6%) and drowsiness (3.2%) as adverse effects[28].

One observational study looked at efficacy and tolerability over six to nine months, including analysis of comorbidities using monthly structured questionnaires, and found that 93% improved 30% or more in at least one symptom category; 47% improved 30% or more in 4 or more symptom categories; 13% improved 30% or more in two symptom categories; and 33% improved 30% or more in one symptom category. The categories were as follows: 1) ADHD 2) behavioral disorders 3) motor deficits 4) autonomy deficits 5) communication and social interaction deficits 6) cognitive deficits 7) sleep problems 8) seizures[29].

Individuals with ASD who used the CBD-rich cannabis extract showed improvement in the following symptoms: self-injury and bouts of anger; hyperactivity; sleep problems, anxiety, restlessness, psychomotor agitation, irritability, aggressiveness, sensory sensitivity, cognition, attention, social interaction, language, perseveration, and depression. Regarding the benefits of the intervention with cannabis, the restlessness symptom was the one that showed the greatest improvement (91%) in relation to the other symptoms studied[30].

Therefore, our results show what the scientific literature demonstrates about its efficacy in hyperactivity, restlessness and psychomotor agitation; in anxiety; in cognition, attention and concentration, in facilitating learning; in nutrition; in social interaction, in children on the autistic spectrum.

Safety of CBD-rich cannabis extract

Currently, cannabidiol is approved by the FDA for the treatment of Dravet and Lennox-Gastaut syndromes, which are related to seizures[31]. Randomized clinical trials have shown that when CBD is added to an anticonvulsant, the frequency of seizures decreases [32,33]. As the scientific literature shows good response to epilepsy, we put, as an exclusion criterion, children who presented, as a comorbidity, epilepsy, in order to analyze specifically the characteristics of cannabis for ASD[34,35].

Numerous pre-clinical studies[36,37] and neuroimaging studies[38] have demonstrated the anxiolytic effects of CBD. A published case series in psychiatric patients found a benefit of CBD for anxiety and sleep[39]. According to the results described, anxiety is indicated as a relevant characteristic associated with ASD, corroborating the scientific literature that presents, in some studies, overall improvement in anxiety[36,37,38].

Researchers advise medical professionals, who encounter young patients using CBD, to discuss its quality and possible adverse effects and drug interactions, which were carefully analyzed in this study[40]. If any subject presented poorly understood symptoms such as fever, diarrhea, vomiting or drowsiness, the adverse effects of CBD oil would not go unnoticed.

In this research, it was found that only three children in the treatment group (9.7%) had adverse effects, which were dizziness and insomnia in one child, colic in one, and weight gain in another. In some studies on adverse effects, the following symptoms were found: sleep disorders, restlessness and nervousness, as well as moderate irritability, diarrhea, increased appetite, conjunctival hyperemia, behavioral problems, decreased cognition, fatigue and aggression/agitation[28,29].

These agents need to be evaluated, over time, of long-term effects of these drugs on development, which remains an open question.

One of the limitations of the study was the coronavirus pandemic, which started during the clinical trial. Since routine is crucial in autistic children's lives, their families were faced with enormous challenges in mitigating the impact of the condition. As the children were divided into six groups of 10 for consultation and product initiation, there was a difference regarding the start of use: those who started before the pandemic (COVID-19) and those who started during the pandemic, as the clinical trial started in January 2020, and countrywide quarantine was implemented in March of the same year in Brazil. For the same reason, laboratory tests could not be performed after the end of the clinical trial.

New randomized, double-blind, placebo-controlled clinical trials, using CBD-rich cannabis extract in higher concentration and even using isolated CBD (phytochemical) for similar analyses, would be an important contribution.

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CONCLUSION

The CBD-rich cannabis extract was found to be safe at the doses used in this study (ranged from 6 to 70 drops/day), given that of all the 31 children who received the extract, only three reported very mild side effects, such as dizziness, insomnia, colic and weight gain. Titration could reach up to 100 drops per day, as directed by the product supplier.

Based on the results obtained, it can be concluded that CBD-rich cannabis extract showed significant improvement in social interaction, anxiety, and psychomotor agitation when compared to children who received the placebo and the CBD-rich cannabis extract did not interfere with the children's sleep quality.

Another important result in this study was an increased number of meals per day from children who received the CBD-rich cannabis extract when compared to the children who received the placebo. This result may be related to the decreased anxiety levels of these children observed after administration of the extract.

Therefore, it is observed that CBD-rich cannabis extract is effective and can be used safely, at least in the short term, in relieving some important symptoms related to ASD in children, such as social interaction, psychomotor agitation and anxiety.

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Two-Way Mixed ANOVA (PSYCHOMOTOR AGITATION)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Time	1	14.70	14.700	9.225	0.00295 **
Group	1	2.99	2.991	1.877	0.17334
time:group	1	0.94	0.937	0.588	0.44465
Residuals	116	184.84	1.593		

---Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Two-Way Mixed ANOVA (NUMBER OF MEALS)

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Time	1	9.63	9.633	4.109	0.045 *
Group	1	4.24	4.236	1.807	0.182
time:Group	1	0.01	0.011	0.005	0.946
Residuals	116	271.99	2.345		

---Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Two-Way Mixed ANOVA (SOCIAL INTERATION)

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Time	1	17.63	17.633	14.133	0.000268***
Group	1	4.49	4.490	3.599	0.60300
time:group	1	0.35	0.349	0.280	0.597960
Residuals	116	144.73	1.248		

---Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Two-Way Mixed ANOVA (ANXIETY)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Time	1	10.21	10.208	5.1989	0.0159*
Group	1	6.87	6.872	4.031	0.0470*
time:group	1	0.51	0.512	0.300	0.5847
Residuals	116	197.73	1.705		

---Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. **(Time: Before or after / Group: cannabis or placebo)**

Two-Way Mixed ANOVA (CONCENTRATION – MILD SEVERITY)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Time	1	5.76	5.558	6.747	0.0124 *
Group	1	2.33	2.327	2.825	0.0993
time:group	1	1.56	1.558	1.891	0.1755
Residuals	48	39.54	0.824		

--- Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. **(Time: before or after / Group: cannabis or placebo).**