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Research Article

Safety and Efficacy of Orally Administered Full-spectrum Medicinal Cannabis Plant Extract 0.08% THC (NTI164) in Children with Autism Spectrum Disorder

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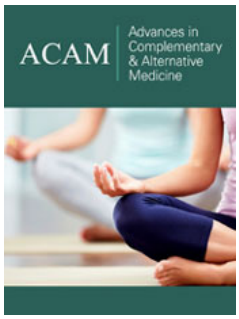

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Abstract

Autism Spectrum Disorder (ASD) is commonly associated with debilitating comorbidities impacting the wellbeing of affected children, young people and their families. Many children with ASD and behavioural problems do not respond well to available medications and may experience adverse side effects. Therefore, it is crucial to develop alternative safe and effective therapies. The improved understanding of the endocannabinoid system, together with emerging evidence for the therapeutic effects of cannabis derivatives in neurodevelopment disorders, has led to an exploration of their use in ASD. This single-group, open-label study assessed the safety and efficacy of a novel oil-based full-spectrum medicinal cannabis plant extract 0.08% Δ-9-Tetrahydrocannabinol (THC) (NTI164) in treating 14 children and young people with ASD symptoms (mean age 13.4 years, range 10-17). Data on the safety profile of NTI164 was collected through biochemical analysis, vital signs, and parent/caregiver and participant reports. The efficacy was assessed through a dose-escalation protocol using a broad range of validated clinical behavioural assessments, caregiver, and participant-reported questionnaires. Following 4 weeks of treatment with NTI164, 93% of participants demonstrated significant overall improvement in ASD-related symptoms compared to baseline, with transient side-effects that did not interfere with their general functioning. In addition, targeted behavioural problems were rated as "much improved" or "very much improved" in 46% of treated patients. More than half of caregivers and participants also reported decreased anxiety symptoms. The findings from this study suggest that NTI164 is well-tolerated and safe, with potential clinical benefits in improving disruptive behaviours and reducing anxiety in young people with ASD-related symptoms. Future longitudinal and well-controlled studies are warranted to develop evidence-based clinical therapies and further evaluate the therapeutic benefit of full-spectrum cannabis extracts in managing ASD core and associated comorbid symptoms.

Trial registration number NCT05516407

Keywords: Anxiety; Autism spectrum disorder; Cannabidiol; *Cannabis*; Cannabinoids; Δ-9-Tetrahydrocannabinol; Inflammation

Abbreviations: AEA: Anandamide; AEs: Adverse Events; ADOS-2: Autism Diagnostic Observational Schedule; ALT: Alanine Aminotransferase; ASC-ASD-C: Anxiety Scale for Children- Autism Spectrum Disorder-Child; ASC-ASD-P: Anxiety Scale for Children-Autism Spectrum Disorder-Parent; ASD: Autism Spectrum Disorder; AST: Aspartate Aminotransferase; CBD: Cannabidiol; CBDA: Cannabidiolic Acid; CBDV: Cannabidivarin; CBGA: Cannabigerolic Acid; CB1/2: Cannabinoid Receptor ½; CGI-C: Clinical Global Impression-Change of Target Behaviour; CGI-CA: Clinical Global Impression-Change in Attention; CGI-I-C:

Clinical Global Impression- Improvement-Clinician; CGI-I-Ca: Clinical Global Impression-Improvement-Caregiver; CGI-S: Clinical Global Impression-Severity of Illness; ECS: Endo Cannabinoid System; MCH: Monash Children's Hospital; NTI164: Full-Spectrum Medicinal Cannabis Plant Extract With 0.08% THC; SD: Standard Deviation; SEM: Standard Error of the Mean; SDSC: Sleep Disturbances Scale for Children; TBL: Total Bilirubin; THC: Δ-9-Tetrahydrocannabinol; ULN: Upper Limit of Normal

Introduction

Autism Spectrum Disorder (ASD) is a group of pervasive neurodevelopmental disorders that appear in early childhood. These disorders are characterised by social functioning deficits, restricted or repeated patterns of behaviour across the lifespan [1], and other comorbidities such as intellectual disability, epilepsy, anxiety, hyperactivity, irritability, and sleep disturbances may also be present [2,3]. These symptoms can significantly impact a person's quality of life and daily functioning [4,5]. Globally, the prevalence of ASD is estimated to range from 0.4% to 1.7% [6]. In the last three decades, the prevalence of young children (under 5 years) diagnosed with ASD has increased by 6.7%, from 2.7 million in 1990 to 2.9 million in 2019 [7]. The families of children with ASD face a substantial financial burden, with recent global estimates indicating 2834 EUR total costs for health-related services combined with indirect societal costs over two months [8]. The estimated lifetime social cost in the United States alone was $7 trillion over the last three decades (1990-2019) and is forecast to increase to an alarming $11-15 trillion in the following decade [9].

The continuing rise in ASD diagnosis is further projected to generate an enormous economic burden on US healthcare for decades and to reach an annual cost estimate of $5.54 trillion by 2060, with substantial potential annual savings of $1.9 trillion through ASD prevention strategies [10]. Despite extensive research, no pharmacological interventions have been shown to be effective for treating ASD core symptom domains, with current evidence-based medication only addressing common comorbidities [11,12]. However, these pharmacological therapies frequently reveal substantial side-effect profiles in children and young people, with significant interindividual variability in efficacy [13] and poor compliance [14]. The complexity of ASD behavioural phenotypes and aetiology resulting from the interaction of multiple genetic and environmental risk factors further limits targeted interventions and the development of effective therapies [15,16]. Therefore, to address core behaviours and comorbid symptoms associated with ASD, targeted therapeutic interventions are urgently needed.

The Endocannabinoid System (ECS), consisting of cannabinoid receptors (CB1 and CB2), endocannabinoids (endogenous bioactive ligands) and metabolic enzymes [17], presents a potential pharmacological target for developing therapeutics in the management of ASD symptoms. The ECS has a crucial role in modulation of neuronal plasticity during development [18] and is associated with social-emotional processes [19] which are impaired in ASD-related phenotypes [20,21]. The central endocannabinoid receptors CB1 and CB2 are predominantly expressed in sensory neurons and immune cells, including microglia [17]. Endogenous cannabinoids are produced as required, activating presynaptic receptors to reduce calcium uptake and inhibit the release of neurotransmitters, consequently influencing a broad range of biological responses [22]. Alterations in ECS functionality have been reported in several preclinical ASD models [23-25] and clinical studies [26-28], suggesting that ASD pathogenesis involves dysregulation of ECS signalling pathways. These findings suggest the Endocannabinoid Anandamide (AEA) and its associated metabolic enzymes may be a potential therapeutic target for ASD-related behavioural impairment.

In animal studies, treatment with inhibitors of the enzyme fatty acid amide hydrolase, which degrades AEA, resulted in increased AEA levels restoring cognitive and social deficits in ASD-related knockout models [29-31]. The increased understanding of ECS signalling concurrent with growing evidence for the therapeutic effects of *cannabis* (*Cannabis sativa L.*) in neurodevelopmental disorders has been the catalyst for extensive research on predominantly two active constituents, Δ-9-Tetrahydrocannabinol (THC) and Cannabidiol (CBD). Recent years have seen a surge in clinical trials of CBD in particular owing to the absence of psychoactive effects and its confirmed favourable safety profile in humans for the treatment of several neurological and psychiatric disorders [32-34]. In addition, accumulating evidence from experimental and animal model studies on the effects of CBD have demonstrated modulation of a broad range of biological responses, including antidepressant [35], antinociceptive [36], anti-seizure [37], immunomodulatory [38] and neuroprotective activities [39]. Cannabinoids are generally well-tolerated, but evidence suggests that the risk of hepatotoxicity may be increased when combined with some medications.

This risk is usually low, dose-dependent, and more likely to occur in individuals with a history of liver disease [40]. Rigorous, controlled clinical studies are needed to determine the efficacy and safety profile of cannabinoids in children and young people. There is growing evidence that using CBD in combination with other compounds found in *cannabis* as a full-spectrum botanical medicine could provide unique therapeutic benefits, as suggested by anecdotal reports and emerging research in the field [41]. This effect, known as the entourage effect, was initially proposed as a regulatory mechanism for the ECS, whereby multiple compounds enhance the activity of endogenous cannabinoids. A recent meta-analysis of observational studies showed that twice as many patients with treatment-resistant epilepsy improved when treated with a multi-compound herbal formulation compared to isolated CBD products, often with a superior safety profile [42]. Promising evidence from preclinical cancer models also supports

the entourage effect, with botanical preparations often producing enhanced therapeutic responses over individual cannabinoids [43-45].

Given that the current single-molecule approach of an individual phytocannabinoid remains the predominant pharmaceutical development strategy [46] for complex diseases such as ASD, multi-target strategies of botanical drugs utilising the entourage effect may be an effective treatment strategy. Evidence from animal studies suggests that CBD positively affects various physiological responses. However, there is limited evidence from clinical trials to support the potential therapeutic benefits of this non-psychoactive phytocannabinoid for paediatric neurodevelopmental disorders [47]. Only three randomised-controlled studies have been published on medicinal cannabis use in children and/or young people with ASD, two of which were short-term [48,49], and the third only reported sleep outcomes [50]. Additionally, most studies on this topic are observational or retrospective in design [51-56]. This single-centre, Phase I/II open-label study aimed to evaluate the safety and efficacy of a novel oil-based full-spectrum medicinal cannabis plant extract with 0.08% THC (NTI164) in children and young people with ASD over 4 weeks of daily treatment.

Material and Methods

Study design and participants

This study was a single-centre, single-group open-label trial to evaluate the safety and efficacy of a novel full-spectrum medicinal cannabis plant extract with 0.08% THC (NTI164) in children and young people with ASD over 4 weeks of daily treatment. The trial enrolled 18 eligible male and female paediatric patients aged 8-17 with Level 2 or 3 ASD based on the Autism Diagnostic Observational Schedule (ADOS-2) criteria [57]. 14 patients (29% female) completed baseline (Week 1) and post-baseline (Week 5) assessments, and all subsequent analyses presented here are of these 14 participants. This study was approved by Monash health human Research Ethics Committee (RES-21-0000-177A) and registered with the Australian and New Zealand Clinical Trial Registry (Trial ID: ACTRN12621000760875).

Study objectives

The primary objective of this study was to evaluate the safety and tolerance of NTI164 in children and young people with ASD throughout the trial. In addition to assessment questionnaires, safety data were collected through laboratory tests, including full blood examination, and liver and renal function tests, performed at screening and at the beginning of Week 5 during hospital visits. The secondary objective was to assess the efficacy of NTI164 in treating ASD-associated symptoms. Efficacy data were collected through physician, parent/caregiver and participant-reported questionnaires at two designated time points (baseline-Week 1 and post-baseline-Week 5). This study explored the effect of NTI164 on core behavioural symptoms of children and young people with ASD, including irritability, hyperactivity, mood, anxiety, sleep disorders, and self-stimulation.

Pharmacological intervention

The chemical composition profile of the investigational product NTI164 consisted of 62% Cannabidiolic Acid (CBDA), 14% CBD, 0.44% Cannabigerolic Acid (CBGA), 0.06% Cannabidivarin (CBDV) and 0.08% THC. The investigational product was provided and dispensed in an amber glass jar as a 53mg/mL oil-based solution ready for oral self-administration at home. During the initial hospital visit (baseline), the participants received the packaged investigational product with instructions. Participants were also given access to an online participant portal to record daily drug administration. Several lines of evidence from controlled trials have shown the effectiveness of CBD at a dose of 20mg/kg in managing seizures [58-60]. Therefore, this dose was used in the current study, with participants starting treatment on a daily dose of 5mg/kg of NTI164 at baseline. To minimise the risk of side effects, the dose was increased by 5mg/kg weekly to reach the maximum tolerated daily dose or 20mg/kg daily. The daily dose was calculated by multiplying the dosage by each patient's weight and then dividing by the concentration of CBDA in the oil (53mg/mL). This provided a total daily volume in mL, which was divided into twice daily doses to further reduce the risk of gastrointestinal upset (from oil). Protocol adherence was assessed by the accountability of returned investigational product and packaging at the end of the 4 weeks.

Outcome measures

Primary outcome measure: The primary endpoint was safety and tolerability of NTI164 treatment as determined by monitoring and assessment through standard laboratory tests of full blood examination, and liver and renal function tests, as well as vital signs in addition to physician and caregiver-reported questionnaires completed at baseline (Week 1) and after completion of four weeks of treatment (Week 5). Adverse Events (AEs) were assessed and evaluated by designated study staff through discussions with the participant at the scheduled study visit after the end of four weeks, as well as through phone calls and clinically significant laboratory results. If an AE was reported during treatment at home, the principal investigator was notified immediately via the online portal. The principal investigator or a designated study staff member promptly followed up on all AEs. Documentation of AEs included details of the event, dates and times, severity, duration, treatment, and possible relationship to the investigational product.

Secondary outcome measure: The efficacy assessments included the administration of both quantitative and qualitative ASD-related questionnaires. The selected questionnaires were specific to various comorbidities associated with ASD. The purpose was to evaluate the impact of NTI164 on symptoms associated with ASD. All scales were administered at baseline (Week 1) and post-baseline (Week 5) (Table 1).

Table 1: Questionnaires used in the current study. ASC-ASD-C=Anxiety Scale for Children-Autism Spectrum Disorder-Child Version; ASC-ASD-P=Anxiety Scale for Children-Autism Spectrum Disorder-Parent Version; CGI-C=Clinical Global Impression-Change of Target Behaviour; CGI-CA=Clinical Global Impression-Change in Attention; CGI-I-C=Clinical Global Impression-Improvement-Clinician; CGI-I-Ca=Clinical Global Impression-Improvement-Caregiver; CGI-S=Clinical Global Impression-Severity of Illness; SDSC=Sleep Disturbances Scale for Children.

Questionnaire	Outcomes Measured
Clinical Global Impression-Improvement, Caregiver (CGI-I-Ca), caregiver-rated [65]	A 7-point scale measuring symptom change from baseline following intervention (NTI164), 4 = no change, <4 = improvement, >4 = worsening.
Clinical Global Impression-Improvement, Clinician (CGI-I-C), clinician-rated [65]	A 7-point scale measuring symptom change from baseline following intervention (NTI164), 4=no change, <4=improvement, >4=worsening.
Clinical Global Impression-Change of Target Behaviour (CGI-C), clinician-rated [65]	A 7-point scale of change in a behaviour from a designated time-point, 1=not at all, 7=very severe problem. Note: target behaviour was chosen by each parent/caregiver, so each participant had a different targeted behaviour (e.g. anxiety, emotional regulation etc).
Clinical Global Impression-Change in Attention (CGI-CA), clinician-rated [65]	A 7-point scale of change in attention from a designated time-point, 1=not at all, 7=very severe problem.
Clinical Global Impression-Severity of Illness (CGI-S), clinician-rated [65]	A 7-point scale of clinician's impression of severity of illness at a given time-point, 1=not at all to 7=among the most extremely ill.
Anxiety Scale for Children-Autism Spectrum Disorder-Parent Version (ASC-ASD-P), caregiver-rated [66]	Measures anxiety symptoms in youth with ASD. Composed of 4 subscales: performance anxiety, uncertainty, anxious arousal, and separation anxiety. A 4-point scale (0=never, 3=always). Subscales sum provides total score.
Anxiety Scale for Children-Autism Spectrum Disorder-Child Version (ASC-ASD-C), participant-rated [66]	Measures anxiety symptoms in children with ASD. Composed of 4 subscales: performance anxiety, uncertainty, anxious arousal, and separation anxiety. A 4-point scale (0=never, 3=always). Subscales sum provides total score.
Sleep Disturbances Scale for Children (SDSC), caregiver-rated [67]	Assesses 6 domains of sleep: disorders of initiating and maintaining sleep, sleep breathing disorders, disorders of arousal, sleep-wake transition disorders, disorders of excessive somnolence, and sleep hyperhidrosis, 5-point scale where 1=never, 5=always (daily). Subscales sum provides total score.

Study procedures

Participants were recruited through Monash Children's Hospital (MCH) in Melbourne, Australia. Potential participants attended a screening visit for assessment based on study selection criteria (see Appendix 1) and were provided with the Participant Information Sheet and Consent Form. After obtaining written informed consent from caregivers, eligible participants underwent a physical assessment, including vital signs, anthropometric measurements, full blood examination, and liver and renal function tests as part of the screening process. In cases requiring sedation for collection of blood samples, nitrous oxide/oxygen was administered by a trained staff member. At this visit, a caregiver survey was obtained concerning the medical and family history of the participant, including current attendance at specific programs or health service utilisation. At the first visit in Week 1 (baseline), participants completed all required questionnaires and the first of the daily dose of 5mg/kg of NTI164 was administered.

Appendix 1: Clinical laboratory values at timepoint 1 (screening) and timepoint 2 (Week 5).

Parameter	Timepoint 1			Timepoint 2			Median Change
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	
Sodium	139	136	142	139	137	141	-1
Potassium	4.3	3.8	4.8	4.4	3.7	4.8	0
Chloride	104	102	108	104	101	106	1
Bicarbonate	25	19	29	25	22	30	0
Urea	4.6	3.1	6.1	4.1	2.6	5.9	0.2
Creatinine	47	35	68	50	36	73	3
Glucose	4.9	3.3	6.3	4.5	2	7.1	-0.1
ALP	287	101	438	280	82	424	-23
GGT	17	11	25	15	8	29	-0.5
ALT	24	5	57	26	6	48	0

Total Bilirubin	11	4	40	9	4	14	0
Haemoglobin	133	115	147	133	119	144	0.2
RBC	4.69	4.1	5.34	4.74	4.36	5.11	0.11
MCV	83	75	90	84	77	90	1.5
MCH	32.4	25.2	86	28.2	25.5	30.8	0.3
MCHC	342	331	349	337	330	348	2
RDW	13.5	12.4	16.7	13.7	12.6	16.9	0.3
WCC	7.6	4.8	14.6	7	4.4	11.4	-0.1
Neutrophils	3.94	1.7	8.69	3.47	1.79	4.7	-0.66
Lymphocytes	2.66	1.45	6.16	2.54	1.15	4.73	-0.035
Monocytes	0.54	0.31	0.79	0.55	0.35	1.05	-0.06
Eosinophils	0.45	0.1	1.24	0.41	0.03	1.14	-0.05
Basophils	0.05	0	0.09	0.06	0	0.1	0.02
Platelets	294	190	413	301	214	422	-13.5

Abbreviations. ALP=Alkaline Phosphatase; ALT=Alanine Transaminase; GGT=Gamma-Glutamyl Transferase; MCH=Mean Corpuscular Haemoglobin; MCHC=Mean Corpuscular Haemoglobin Concentration; MCV=Mean Corpuscular Volume; RBC=Red Blood Cell; RDW=Red Cell Distribution Width; WCC=White Cell Count.

The participant was monitored for 2 hours by clinical staff for any immediate AEs. Participants who did not report any AEs were dispensed with 4 weeks supply of NTI164 and instructed to administer the daily volume orally in two divided doses (i.e. a morning and evening dose). Instructions were provided on administering increasing doses of the product to a maximum of 20mg/kg/day from home. Caregivers were trained to use the online participant portal to record drug administrations, report AEs, and complete questionnaires from home. At the Week 5 visit (post-baseline), participants underwent the same set of laboratory tests and physical assessments as baseline, and completed all specified post-baseline questionnaires. To ensure adherence to study protocol and accountability, returned product and packaging were collected and recorded at this visit.

Statistical analysis

The safety and tolerability of the investigational product was assessed by recording the frequency and severity of reported AEs by all treated participants. The distribution was analysed using patient demographics, laboratory values, and vital signs. Given the small sample size of this study, descriptive statistical analyses of the mean score, mean change, and Standard Deviation (SD) were performed on quantifiable outcome measures. Paired t-tests were used (RStudio software) to assess the statistical significance of the analysed data sets of Week 5 compared to Baseline. The final statistical analyses included all participants completing the initial 4-week treatment phase (n=14).

Result

Participant characteristics

14 of the 18 participants enrolled in the study completed the full 4-week treatment period. 1 patient withdrew before treatment began, and 3 patients discontinued during treatment. Most treated patients identified as male (9/14), Caucasian (12/14), and had an average age of 13.4 years (ranging from 10-17 years). All active patients were diagnosed with ASD Levels 2 or 3 and were evaluated at baseline as "Mildly ill" (4 patients), "Moderately ill" (4 patients), "Markedly ill" (3 patients), or "Severely ill" (3 patients) according to the CGI-S. The average maximum tolerated dose for treated patients was 16.7mg/kg/day. 64% of patients could tolerate the maximum dose of 20mg/kg/day, while 36% of patients tolerated a maximum daily dose ranging between 6mg/kg/day and 19mg/kg/day.

Safety and tolerability

8 participants experienced 10 mild-moderate AEs, totalling 17 adverse reports during treatment (Table 2). The most frequent side-effect was gastrointestinal symptoms reported by a third of participants (36%). None of these side effects were severe enough to significantly interfere with the patient's functioning (determined by the treating physician). Most patients were advised to take rest to treat these AEs. No serious AEs or deaths related to the investigational drug were reported during the study. The safety data indicated that NTI164 at 5, 10, 15 and 20mg/kg administered in two daily doses is safe and well-tolerated in this study population. Laboratory values provided further safety support (Appendix 1). No changes were observed in patients' full blood examination, liver function or kidney function tests. There were no changes observed to vital signs.

Table 2: Reported AEs during the trial. ** This participant was withdrawn from the study.

Adverse Event	Total No. of Reports	Frequency (%)	Treatment
Abdominal pain	3	14%	Rest
Lack/loss of appetite	3	14%	None
Diarrhoea	2	9%	None
Vomiting	2	9%	None
Lethargy	2	9%	Rest
Hypoglycaemia	1	5%	None
Rashes**	1	5%	Zyrtec for itchiness
Itchiness without rash	1	5%	Warm shower
Sore Throat	1	5%	Rest
Upper Respiratory Tract Infection	1	5%	Treated by doctor

Analysis of therapeutic efficacy

Clinical Global Impression-Severity (CGI-S): Several CGI scales were implemented to assess Severity of Illness and Improvement following an intervention (NTI164) in this population (Table 3). For detailed statistical information, see Appendix 2. The CGI-S scale was used to assess global improvement, the severity of illness, and the efficacy index based on drug effect only. Of the 14 treated patients, 13 (93%) showed symptomatic improvement. 9 (64%) of these patients showed a global improvement of 'much improved', 4 (29%) showed 'minimally improved', and only 1 patient (7%) had 'no change' (Table 3). At baseline, the average rating for the severity of illness was 4.4, which was significantly reduced to an average rating of 2.4 after 28 days of NTI164 treatment ($p<0.0001$, Figure 1A). The CGI-I-Ca and CGI-I-C scales were used to assess overall improvement following NTI164 as rated by the participants' caregiver and clinician, respectively. When rated by the primary caregiver, 12 (86%) patients improved (Table 3). 1 (7%) patient was reported to be 'very much improved', 7 (50%) were 'much improved', 4 (29%) 'minimally improved', and 2 (14%) reported 'no change'.

Table 3: Symptom change scores for each Clinical Global Impression (CGI) scale. CGI-C=Clinical Global Impression-Change of Target Behaviour; CGI-CA=Clinical Global Impression-Change in Attention; CGI-I-C =Clinical Global Impression-Improvement-Clinician; CGI-I-Ca=Clinical Global Impression-Improvement-Caregiver; CGI-S=Clinical Global Impression-Severity of Illness. N=14.

CGI Assessment	Symptom Change from Baseline			
	1. Very Much Improved	2. Much Improved	3. Minimally Improved	4. No Change
CGI of Severity of Illness (CGI-S)	0	9 (64%)	4 (29%)	1 (7%)
CGI of Improvement for the Caregiver (CGI-I-Ca)	1 (7%)	7 (50%)	4 (29%)	2 (14%)
CGI of Improvement for the Clinician (CGI-I-C)	1 (7%)	4 (29%)	7 (50%)	2 (14%)
CGI of Change in Target Behaviour (CGI-C)	2 (14%)	4 (29%)	3 (21%)	5 (36%)
CGI of Change in Attention (CGI-CA)	0	3 (21%)	4 (29%)	7 (50%)

Appendix 2: Statistic analyses of clinical surveys used in the current study.

Questionnaire	Subdomain	Statistics				
		P value	95% CI	T value	SD	SEM
CGI-S	-	<0.0001	1.23-2.63	5.98	B: 1.15 PB: 0.65	B: 0.31 PB: 0.17
CGI-I-Ca	-	<0.0001	1.1-1.99	6.57	B: 0 PB: 2.5	B: 0 PB: 0.23
CGI-I-C	-	<0.0001	0.81-1.76	5.83	B: 0 PB: 0.83	B: 0 PB: 0.22

CGI-C	-	<0.0001	2.13-4.02	7.01	B: 1.03 PB: 1.12	B: 0.27 PB: 0.3
CGI-CA	-	0.0022	0.59-2.13	3.8	B: 0.84 PB: 0.83	B: 0.23 PB: 0.22
ASC-ASD-P	Performance anxiety	0.0306	0.11-1.89	2.45	B: 4.51 PB: 3.72	B: 1.25 PB: 1.03
	Anxious arousal	1	1.54-1.54	0	B: 3.93 PB: 3.28	B: 1.09 PB: 0.91
	Separation anxiety	0.2301	-0.5-1.89	1.26	B: 3.93 PB: 3.28	B: 1.09 PB: 0.91
	Uncertainty	0.0207	0.38-3.78	2.66	B: 6.12 PB: 4.98	B: 1.7 PB: 1.38
	Total score	0.0297	0.44-7.10	2.47	B: 15.67 PB: 13.81	B: 4.34 PB: 3.83
ASC-ASD-C	Performance anxiety	0.2779	-0.68-2.14	1.15	B: 3.67 PB: 3.79	B: 1.11 PB: 1.14
	Anxious arousal	0.3531	-3.29-1.29	0.97	B: 3.87 PB: 3.46	B: 1.17 PB: 1.04
	Separation anxiety	0.0745	-0.17-3.08	1.99	B: 3.82 PB: 4.44	B: 1.15 PB: 1.34
	Uncertainty	0.1214	-0.37-2.74	1.69	B: 5.75 PB: 5.94	B: 1.73 PB: 1.79
	Total score	0.1937	-1.42-6.14	1.39	B: 11.68 PB: 15.06	B: 3.52 PB: 4.54
SDSC	Disorders of initiating and maintaining sleep	0.5307	-2.19-4.04	0.65	B: 7.57 PB: 6.49	B: 2.1 PB: 1.8
	Sleep breathing disorders	0.2132	-0.3-1.23	1.31	B: 3.07 PB: 2.3	B: 0.85 PB: 0.64
	Disorders of arousal	0.7762	-0.5-0.65	0.29	B: 1.57 PB: 1.24	B: 0.44 PB: 0.34
	Sleep-wake transition disorders	0.839	-1.46-1.77	0.21	B: 4.59 PB: 3.15	B: 1.27 PB: 0.87
	Disorders of excessive somnolence	0.503	-2.88-1.49	0.69	B: 3.5 PB: 3.79	B: 0.97 PB: 1.05
	Sleep hyperhidrosis	0.3033	-0.32-0.93	1.08	B: 1.18 PB: 0.87	B: 0.33 PB: 0.24
	Total score	0.7274	-6.29-8.75	0.36	B: 17.87 PB: 13.61	B: 4.96 PB: 3.77

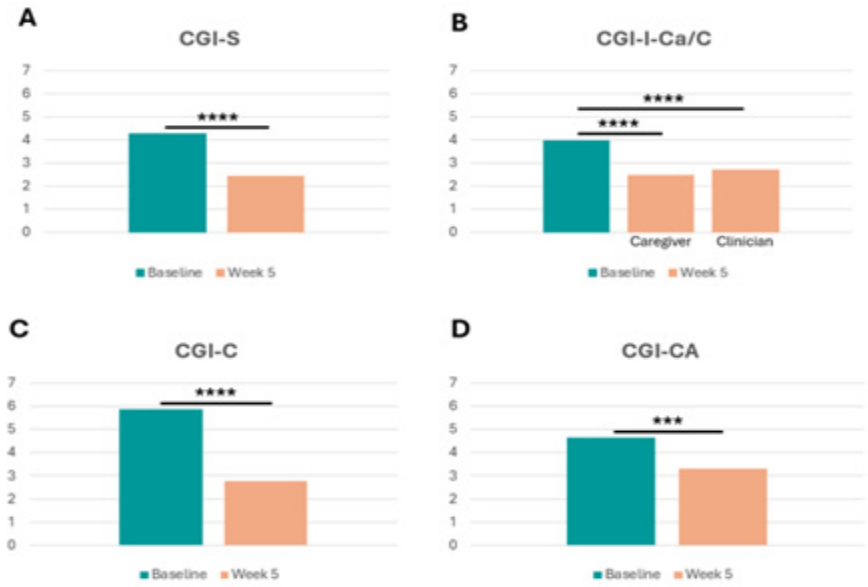

Figure 1: CGI scales following 4 weeks of NTI164 administration (Week 5) compared baseline (Week 1). NTI164 significantly improves clinical symptoms of ASD. (A) CGI-S-Mean score of 4.4 at baseline reduced to 2.4; (B) CGI-I-Ca/C-Mean CGI-I-Ca score of 4 at baseline reduced to 2.5, mean CGI-I-C score reduced from 4 at baseline to 2.7; (C) CGI-C - mean score was reduced from 4 at baseline to 2.8; (D) CGI-CA-Mean score was reduced from 4 at baseline to 3.3. Paired t-test, ***p<0.001, ****p<0.0001, n=14.

The mean CGI-I-Ca score was reduced (i.e. improved) from 4 at baseline (i.e. 'no change') to 2.5 at Week 5 (p<0.0001, Figure 1B). When rated by the treating physician, 1 (7%) patient was rated as 'very much improved', 4 (29%) as 'much improved', 7 (50%) as 'minimally improved', and 2 (14%) as 'no change'. The mean CGI-I-C score was significantly reduced from 4 (i.e. 'no change') at baseline to 2.7 at Week 5 (p<0.0001, Figure 1B). The CGI-C scale was used to assess change in a specific behaviour, chosen by the primary caregiver for each participant (e.g. anxiety, emotional regulation). After 4 weeks of NTI164 administration, target behaviours were rated as 'very much improved' in 2 (14%) patients, 'much improved' in 4 (29%) patients, 'minimally improved' in 3 (21%) patients, and 5 (36%) patients were reported as showing 'no change' (Table 3). The mean CGI-C score was significantly reduced (i.e. improved) from 4 (i.e. 'no change') at baseline to 2.8 at Week 5 (p<0.0001, Figure 1C). The CGI-CA scale was used to assess changes in attention after 4 weeks of NTI164 administration compared to baseline. At Week 5 attention was reported to be 'much improved' in 3 (21%) patients, 'minimally improved' in 4 (29%) patients, and 'no change' reported in 7 (50%) patients (Table 3). The mean CGI-CA score was significantly reduced (i.e. improved) from 4 (i.e. 'no change') at baseline to 3.3 at Week 5 (p=0.002, Figure 1D). At the end of the study, 14% of active patients receiving daily treatment with NTI164 demonstrated the second highest possible efficacy index of 2, indicating marked therapeutic effects with side effects that did not significantly interfere with functioning (Table 4). Clinicians rated NTI164 to have a moderate therapeutic effect (efficacy index of 5 or 6) in 72% of treated patients, with half having no side effects and the other half having side effects that did not significantly interfere with their functioning. A minimal therapeutic effect (efficacy index of 9) was observed in 7%, and an unchanged therapeutic effect (efficacy index of 13) in 1 participant.

Table 4: Clinical global impression-therapeutic effect after 28 days of treatment.

Therapeutic Effect		Side Effects			
		None	Do not Significantly Interfere with Patient's Functioning	Significantly Interfere with Patient's Functioning	Outweighs Therapeutic Effect
Marked	Vast improvement. Complete or nearly complete remission of all symptoms.	01	02	03	04
Moderate	Decided improvement. Partial remission of symptoms.	05	06	07	08
Minimal	Slight improvement which doesn't alter status of care of patient.	09	10	11	12
Unchanged or worse		13	14	15	16

Anxiety symptoms: To measure anxiety symptoms in treated participants with ASD, the Anxiety Scale for Children, both -Parent (ASC-ASD-P) and -Child (ASC-ASD-C) versions were used the total score ranged from 6 to 55 and 3 to 50 out of a possible maximum score of 72 for the ASC-ASD-P and ASC-ASD-C version, respectively. For detailed statistical analysis, see Appendix 2. The total score of the ASC-ASD was reduced in more than half for both the -Parent (62%) and Child (54%) scales after four weeks of treatment. During the same period, nearly a quarter of caregivers (23%) and a third of participants (36%) reported increased levels of anxiousness, while 15% and 9% of caregivers and participants reported no change, respectively. The ASC-ASD-P Total Score saw the mean score significantly reduce (i.e. improve) from 30 at baseline to 25.9 at Week 5 (p= 0.0297, Figure 2A). When analysed by subdomain, Performance Anxiety reduced from (mean) 7.15 to 6.15 (p=0.03), and Uncertainty reduced from (mean) 13.46 to 11.38 (p=0.02). Anxious Arousal and Separation Anxiety did not see any statistically significant changes following NTI164 administration. The ASC-ASD-C did not report any significant changes during the treatment period, however, there was a positive trend towards improvement in Separation Anxiety (p=0.07, Figure 2B).

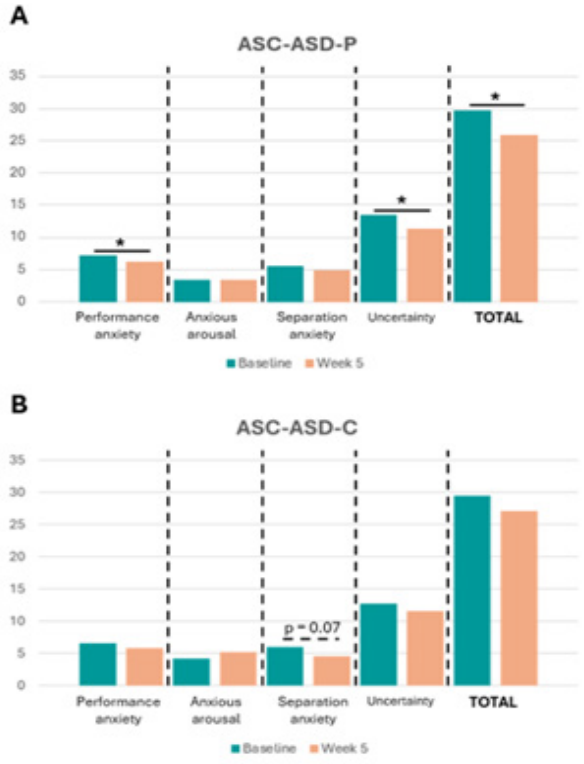

Figure 2: ASC-ASD-P and ASC-ASD-C scales following 4 weeks of NTI164 administration (Week 5) compared baseline (Week 1). NTI164 significantly reduces anxiety in ASD according to primary caregivers. (A) ASC-ASD-P-mean score in specific domains and overall anxiety, which was reduced from (mean) 30 at baseline to 25.9 at Week 5. (B) ASC-ASD-C-mean score in specific domains, no changes were reported. Paired t-test, *p<0.05, n=14 for ASC-ASD-P, n=10 for ASC-ASD-C.

Sleep quality and sleep disturbances: To assess changes to sleep quality, the SDSC questionnaire was used. Of the 14 study participants, 11 participants completed the sleep assessment before and after 4 weeks of NTI164 administration. Although the total sleep score decreased from baseline to post-treatment, it was not statistically significant (Figure 3). There were also no statistically significant changes seen in subdomains measured.

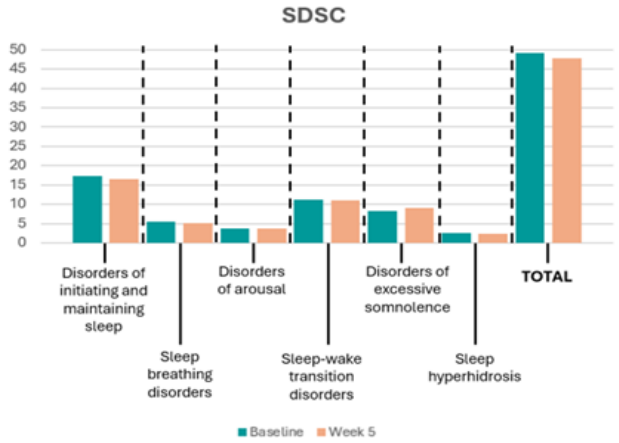

Figure 3: SDSC at baseline and following 4 weeks of NTI164 administration. No significant differences were seen in any subdomains or in overall total score. Paired t-test, n=11.

Discussion

The current pharmacological and behavioural interventions for ASD are limited in treating a considerable portion of paediatrics with ASD-related symptoms due to lack of efficacy or side effects that interfere with daily functioning. Therefore, there is an urgent need for a more concerted effort in clinical research to develop effective and safer therapies to address ASD core and associated comorbid symptoms. While the available evidence from controlled studies on the role of medicinal Cannabis sativa in the management of paediatrics with ASD is limited, a large body of evidence from preclinical studies has demonstrated the positive effects of cannabinoids in the modulation of a wide range of biological responses, in particular neurodevelopmental processes. Furthermore, emerging evidence from recent observational studies [52-54,56] has indicated that a synergistic and entourage pharmacological effect of Cannabis sativa derivatives can contribute to improved ASD-related symptoms and comorbidities in the paediatric population.

Therefore, this study sought to provide evidence for the safety and efficacy of NTI164, a full spectrum botanical product, per the entourage effect in the management of ASD core and comorbid symptoms in a paediatric population. Our findings demonstrate that NTI164 is safe and well-tolerated in children and young people up to 20mg/kg daily dose, with transient side-effects that did not significantly interfere with the general functioning of treated patients. Furthermore, NTI164 exhibited statistically significant efficacy in improving ASD core and associated symptoms after

four weeks of daily treatment. Following NTI164 treatment, targeted behavioural problems and overall clinical impressions were 'much improved' or 'very much improved' in nearly half of the participants. More than half of the caregivers and participants also reported consistent improvements in anxiety levels amongst participants. Based on these promising findings, we have initiated an extended treatment open-label trial for 57 weeks to assess long-term NTI164 safety and efficacy in children and young people with ASD and related comorbidities.

Impact of full-spectrum cannabis extract on ASD core symptoms

The safety profile and tolerability of NTI164 was favourable in the patients who completed treatment, consistent with previous clinical studies that used whole plant medicinal cannabis extract for the treatment of children and young people with ASD-related symptoms [48,51-57]. The most frequent side-effects in the study cohort were related to gastrointestinal symptoms, which were transient and improved with rest by the end of the treatment phase. Of note, the majority of previous clinical studies using full-spectrum cannabis extract for the treatment of children and young people with ASD reported mild or transient adverse events frequently related to sleep disturbances [48,49,51-54], anxiety or irritability symptoms [48, 51,54,56]-adverse events which were not found in this study. This may be due to different strains with variable composition of phytocannabinoids and higher doses of THC than NTI164-a full-spectrum medical cannabis extract with negligible THC (0.08%). Alternatively, side-effects not observed in this study might be due to other studies including primarily children diagnosed with severe ASD, since sleep disturbances have been reported as a side-effect of isolated CBD treatment in children with severe behavioural problems [61]. In addition, several studies have also found that the severity of ASD core symptoms is associated with the severity of sleep disturbances in younger children [50,62,63].

Compared to other clinical studies that included children as young as 4 years old, active patients in this study were much older (10-17 years). The effect of different compositions of phytocannabinoids and THC levels in full-spectrum extracts on the severity of ASD symptoms in younger children, therefore, requires further investigation. To the best of our knowledge to date, this is the first study of full-spectrum cannabis extract treatment in paediatrics with ASD to report subjective measures of AEs together with objective measures of biochemical analysis and vital signs following treatment. In a prospective cohort study of biochemical safety analysis of an oil-based full-spectrum cannabis product, all parameters were within the normal range in children and young people with ASD after three months of treatment [64]. However, this study did not report an evaluation of AEs or vital signs following treatment. Notably, a recent randomised controlled feasibility study reported no significant abnormal biochemical test results after eight weeks of treatment with isolated CBD in children and young people with severe behavioural problems and intellectual disabilities [61]. Our study supports these findings of full-spectrum cannabis products being well-tolerated, with no abnormal laboratory values observed during the treatment period.

In our study, overall daily treatment with NTI164 for four weeks significantly improved ASD core symptoms in 93% of active patients, with nearly two-thirds of participants rated by clinicians to exhibit at least a moderate effect following treatment (Figure 1), (Table 4). Similarly, several cohort studies in children and young people using full-spectrum products have demonstrated significant overall improvement in ASD symptoms reported by parents/caregivers [51-53,55] and clinicians [54,56]. Specifically, studies have shown that almost half of the treated children and young people with ASD showed reduced disruptive behaviour, which is consistent with other studies that have demonstrated improvement in behavioural problems ranging from 32% to 90%, including adaptive behaviours, self-injury, and hyperactivity [49,52,56]. Social interaction and communication have also been found to significantly improve following treatment with full-spectrum cannabis extract in children and young people with ASD-related symptoms [51,54-56]. In addition, two randomised controlled trials have shown that cannabinoid treatment compared to placebo in children and young people was safe with acceptable tolerability and effective at reducing ASD-associated disruptive behaviours [48,49]. However, compared to pure cannabinoids, there was no clear difference with improvements observed for treatment with whole-plant extract after 12 weeks. The clinical benefits of the entourage effects of our cannabis strain compared to purified cannabinoids remains to be further explored in longitudinal controlled studies.

Impact of full-spectrum cannabis extract on ASD-related comorbidities

In addition to improvements in behavioural outcomes, a recent systematic review of the evidence for medicinal cannabis in ASD treatment found benefits for a broad range of comorbidities, including cognition, anxiety and sleep disturbances in adults and young people [65]. Consistent with previous studies [51-53], we also found that after four weeks of treatment with full-spectrum cannabis extract, anxiety symptoms were reduced in more than half of our cohort based on both parent/caregiver and participant-reported anxiety questionnaires. In particular, the parent/caregiver-rated total scores and the Performance Anxiety and Uncertainty subscales significantly improved for children and young people with ASD. However, the child/young people-rated outcome measures were not significantly improved despite the direction and magnitude of change, suggesting reduced anxiety symptoms in half of the treated patients. This rating discrepancy might be due to differences in how anxiety is perceived by children and young people with ASD and their parents or caregivers. This divergence in ratings may also be influenced by the severity of ASD symptoms, as higher severity levels seem to be linked to a trend where parental or caregiver rating surpass those provided by the children and young people in assessing anxiety levels [66]. Nevertheless, this finding highlights the clinical implications of self-report ratings to inform areas of concern for continued assessment of anxiety symptoms in children and young people with ASD.

While previous prospective cohort studies have reported treatment with whole plant cannabis extract of various strains significantly improved sleep outcomes in children and young people with ASD [52-54], a recent randomised controlled trial did not find any difference between cannabinoid treatment and placebo in sleep parameters after 12 weeks [50]. We also did not find significant improvement in any of the sleep domains as measured by a validated sleep questionnaire. These inconsistencies could be due to differences in treated participant characteristics, especially severity of ASD, age, instruments used to measure sleep, or strain of cannabis with various phytocannabinoid composition. In addition, since the previous studies that reported improved sleep outcomes were over several months, this may indicate the need for a longer period before any benefit in sleep quality can be observed from treatment with cannabinoid extract. Collectively, these findings provide additional support for the safety and efficacy of full-spectrum products, such as NTI164, and promising entourage effects of cannabinoids in the treatment of children and young people with ASD-related symptoms [67-73].

Limitations

There were several limitations in this study. As our study was an open-label design to collect preliminary data on the safety and efficacy of NTI164 treatment of symptoms in children and young people with ASD, no control group was included. However, the addition of objective measures of vital signs and biochemical analysis to subjective reports of adverse events further strengthened the NTI164 safety profile in treated participants with ASD. Nevertheless, further research for long-term evaluation of safety data of NTI164 botanical product for the treatment of children and young people with ASD symptoms, especially in terms of interaction with concomitant medications, is needed. Given that the sample size was small, evaluation of the impact of NTI164 treatment on subgroups of children and young people with various ASD severity was not possible. Although data was collected on concomitant medications that were prescribed for ASD-related comorbidities, this study was not powered to determine the impact of chronic medication on treatment response. Also, we only used subjective measures of sleep outcomes, and future studies should include actigraphy or polysomnography together with sleep diaries as objective measurement of sleep quality.

Conclusion

This open-label study provides preliminary safety and efficacy data on a novel botanical formulation of phytocannabinoids in the treatment of symptoms in children and young people with ASD and associated comorbidities. Results indicate that NTI164 is safe and well-tolerated in this population, with significant efficacy in improving disruptive behaviours and reducing anxiety in these paediatric patients. The findings of this study further contribute to the accumulating evidence in favour of whole plant cannabis extracts, with most participants reporting a clinically meaningful change in ASD core symptoms as well as associated anxiety. Despite the small sample size, objective safety analysis together with converged evidence from standardised clinician, parent/caregiver and participant-reported questionnaires of overall improvement, strengthen the evidence for the benefits of full-spectrum cannabis extract in the treatment of young people with ASD-related symptoms. Furthermore, these results are consistent with other prospective cohort studies of full-spectrum cannabis extract and support further evaluation of the clinical benefits of whole plant extracts in rigorous placebo-controlled trials of longer duration in this patient population.

Supplementary

Inclusion/exclusion criteria

Subject inclusion criteria:

a) Participant is aged 8 years to 17 years (inclusive).

b) Participant is at a healthy weight at the discretion of the Principal Investigator.

c) Parents or caregivers can give informed consent for participation in the trial with assent from individuals with autism.

d) Participants can comply with trial requirements.

e) According the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) criteria the participant has a diagnosis of Level 2 or 3 Autism Spectrum Disorder (ASD) confirmed by Autism Diagnostic Observational Schedule (ADOS-2) criteria.

f) All treatments including medications and therapies for ASD related symptoms have been stable for 4 weeks before enrolment and for the duration of the trial.

g) Participants must be able to swallow liquid.

h) Consent giver must be able to understand the requirements of the study.

Subject exclusion criteria:

a) Current diagnosis of bipolar disorder, psychosis, schizophrenia, schizoaffective disorder, or active major depression.

b) Has a diagnosis other than ASD that dominates the clinical presentation (e.g., Attention Deficit Hyperactivity Disorder [ADHD]).

c) Has a degenerative condition.

d) Changes in anticonvulsive therapy within the last 12 weeks.

e) Taking omeprazole, lansoprazole, tolbutamide, warfarin, sirolimus, everolimus, temsirolimus, tacrolimus, clobazam, repaglinide, pioglitazone, rosiglitazone, montelukast, bupropion, or efavirenz.

f) Currently using or has used recreational or medicinal cannabis, cannabinoid-based medications (including Sativex®, or Epidiolex®) within the 12 weeks prior to screening and is unwilling to abstain for the duration of the trial.

g) Participant has any known or suspected hypersensitivity to cannabinoids or any of the excipients.

h) Participant has moderately impaired hepatic function at screening, defined as serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST)>2×Upper Limit of Normal (ULN) or Total Bilirubin (TBL)>2×ULN. This criterion can only be confirmed once the laboratory results are available; participants enrolled into the trial who are later found to meet this criterion must be screen-failed.

i) Participant is male and fertile (i.e., after puberty unless permanently sterile by bilateral orchidectomy) unless willing to ensure that they use male contraception (condom) or remain sexually abstinent during the trial and for 12 weeks thereafter.

j) Participant is female and with childbearing potential (i.e., following menarche and until becoming postmenopausal for ≥12 consecutive months unless permanently sterile by hysterectomy, bilateral salpingectomy, or bilateral oophorectomy) unless willing to ensure that they use a highly effective method of birth control (e.g., hormonal contraception, intrauterine device/hormone-releasing system, bilateral tubal occlusion, vasectomized partner, sexual abstinence) during the trial and for 12 weeks thereafter.

k) Female participant who is pregnant (positive pregnancy test), lactating or planning pregnancy during the course of the trial or within 12 weeks thereafter.

l) Participant had brain surgery or traumatic brain injury within 1 year of screening.

m) Participant has any other significant disease or disorder which, in the opinion of the Investigator, may either put the participant, other participants, or site staff at risk because of participation in the trial, may influence the result of the trial, or may affect the participant's ability to take part in the trial.

n) Any abnormalities identified following a physical examination of the participant that, in the opinion of the Investigator, would jeopardize the safety of the participant if they took part in the trial.

o) Any history of suicidal behaviour (lifelong) or any suicidal ideation of type 4 or 5 on the Columbia-Suicide Severity Rating Scale (C-SSRS) in the last 4 weeks or at screening or randomisation.

p) Participant has donated blood during the past 12 weeks and is unwilling to abstain from donation of blood during the trial.

q) Participant has any known or suspected history of alcohol or substance abuse or positive drugs of abuse test at screening (not justified by a known concurrent medication).

r) Participant has previously been enrolled into this trial.

s) Participant has plans to travel outside their country of residence during the trial, unless the participant has confirmation that the product is permitted in the destination country/state.

Conflict of Interest

BK, YO, and EI were employees of a funding body of this research, Fenix Innovation Group Pty Ltd., during the period of this study and may hold stock or options in the company. BK, YO, and EI did not participate in data collection or analysis. The remaining authors declare no conflicts of interest. Data analysis was performed by an independent statistician.

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