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QUATRE FOLIC FOR REDUCING SEVERITY OF AUTISTIC SYMPTOMS IN CHILDREN LESS THAN 5 YEARS: A RANDOMISED CONTROLLED TRAIL

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Abstract

Background: Autism spectrum disorder (ASD) is a complex neurodevelopmental condition with multifactorial etiology, including possible disruptions in folate metabolism. **Objective:** To evaluate the effectiveness of Quatre Folic supplementation in reducing the severity of autistic symptoms in children under five years of age, as measured by pre- and post-intervention CARS-II scores. **Methods:** This study was designed as a single-centre, hospital-based, prospective, parallel-group, double-blinded, randomized controlled trial conducted in the Department of Paediatrics at Saveetha Institute of Medical and Technical Sciences, Chennai, over a period of five months from January to May 2025. **Results:** The baseline characteristics of participants in the Quatre Folic (QF) and Standard Treatment (ST) groups were comparable in terms of age, gender distribution, anthropometric measures, haemoglobin levels, and CARS-II scores, indicating well-balanced groups. After 12 weeks of intervention, the QF group exhibited a significantly greater reduction in autism severity, with a mean decrease in CARS-II score of 3.02 ± 1.17 compared to 1.05 ± 0.75 in the ST group ($p < 0.001$). The post-intervention mean CARS-II score in the QF group was 29.82 ± 1.89, significantly lower than the ST group's 32.36 ± 1.76 ($p = 0.001$), reflecting meaningful clinical improvement. The QF group also showed a significant increase in haemoglobin levels from 11.57 ± 0.90 to 11.77 ± 0.80 g/dL, whereas the ST group remained unchanged (11.13 ± 1.10 to 11.12 ± 1.13 g/dL), with a between-group mean difference of 0.22 g/dL ($p = 0.003$). Changes in weight-for-age Z-scores were minimal, with the QF group improving from −1.05 ± 0.93 to −0.97 ± 0.94, while the ST group slightly declined from −0.55 ± 0.77 to −0.57 ± 0.79; however, this difference was not statistically significant ($p = 0.182$). **Conclusion:** Quatre Folic supplementation significantly reduced autism severity and improved haemoglobin levels in children under five with mild to moderate ASD. These findings support its potential as an effective adjunctive therapy in early autism management.

Keywords: Autism Spectrum Disorder, Quatre Folic, CARS-II Scale, L-methylfolate, Randomized Controlled Trial, Paediatric Neurodevelopment

INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by deficits in social communication and interaction, along with restricted, repetitive patterns of behaviour, interests, or activities.(1) Globally, the prevalence of ASD has been increasing, with recent estimates suggesting that approximately 1 in 100 children are affected.(2) In India, the reported prevalence varies from 0.15% to 1.01%, highlighting the emerging public health concern posed by ASD in the paediatric population.(3, 4)

The pathogenesis of ASD is multifactorial, involving genetic, epigenetic, and environmental components. Among these, alterations in folate metabolism have garnered increasing attention.(5) Folate plays a crucial role in neurodevelopment, particularly in DNA methylation, neurotransmitter synthesis, and the regulation of oxidative stress.(6) Children with ASD have been shown to exhibit abnormalities in folate-related pathways, including

impaired transport of folate across the blood–brain barrier due to the presence of folate receptor autoantibodies (FRAAs).(7) These findings have paved the way for research on folate-based therapeutic interventions in ASD.

Recent studies have indicated that supplementation with folate derivatives may ameliorate certain symptoms associated with autism.(8) Notably, 5-methyltetrahydrofolate (5-MTHF), the biologically active form of folate, bypasses the need for enzymatic conversion, making it particularly useful in individuals with polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene.(9) Quatre Folic®, a fourth-generation folate derivative comprising the glucosamine salt of (6S)-5-methyltetrahydrofolate, has superior stability, bioavailability, and cellular uptake compared to earlier folate formulations.(10) Given these pharmacokinetic advantages, Quatre Folic presents a promising alternative to conventional folic acid in addressing folate-related metabolic dysfunction in ASD. Evidence from clinical trials suggests that supplementation with folinic acid or 5-MTHF may result in behavioural improvements in subsets of children with ASD, particularly those with FRAAs or MTHFR polymorphisms.(11, 12) However, studies specifically evaluating the efficacy of Quatre Folic in young children with autism are scarce. Moreover, few studies have utilized standardized rating scales, such as the Childhood Autism Rating Scale, Second Edition (CARS-II), to quantify symptom severity pre- and post-intervention. Against this background, the objectives of the present study were to estimate the effect of Quatre folic supplementation in reducing severity of autistic symptoms among children less than 5 years of age; and to compare children pre and post supplementation of Quatre folic using CARS-II scale.

MATERIALS AND METHODS

This study was designed as a single-centre, hospital-based, prospective, parallel-group, double-blinded, randomized controlled trial aimed at assessing the efficacy of Quatre folic supplementation in reducing the severity of autistic symptoms in children under five years of age. The research was conducted in the outpatient and inpatient departments of the Department of Paediatrics at Saveetha Institute of Medical and Technical Sciences, Chennai, over a period of five months from January to May 2025. Prior to initiation, ethical clearance was obtained from the Institutional Human Ethics Committee (IHEC) of Saveetha Institute of Medical and Technical Sciences. All participants and their legal guardians received a Participant Information Sheet (PIS) in the local language, detailing the nature, purpose, procedures, potential risks, and benefits of the study. The content of the PIS was explained verbally to ensure comprehension. Written informed consent was obtained from the guardians before enrolment into the study.

Children aged between 2 and 5 years who were diagnosed with ASD based on DSM-5 criteria and a CARS-II (Childhood Autism Rating Scale, Second Edition) score indicating mild to moderate autism were included in the study. Exclusion criteria were children with severe autism (CARS-II score >37), those currently receiving or having received folate or multivitamin supplementation in the past three months, children with chronic systemic illness, known metabolic or genetic disorders, or those with a history of seizures requiring antiepileptic medication. The sample size was determined based on preliminary effect size estimates from previous studies assessing behavioral interventions in ASD. With an anticipated effect size (Cohen's d) of 0.9, a significance level (α) of 0.05, and a power of 80%, a minimum of 13 participants per group was required. To account for potential dropouts, the final sample size was set at 15 participants in each group, leading to a total of 30 children enrolled and randomized into two groups: the intervention group receiving Quatre folic (Group QF) and the control group receiving standard treatment (Group ST).

Participants were allocated to the Group QF or ST using computer-generated randomization in a 1:1 ratio. Allocation concealment was maintained using opaque, sealed envelopes, and both participants and investigators involved in outcome assessment were blinded to group allocation. Quatre folic was administered once daily for a period of 12 weeks in the intervention group. Data were collected at two time points: baseline (prior to intervention) and at the end of 12 weeks. At both time points, the severity of autistic symptoms was assessed using the CARS-II. CARS-II is a validated and widely used diagnostic tool for autism severity assessment, comprising 15 items rated on a 4-point scale based on direct observation and parental reports. The total score ranges from 15 to 60, with scores between 30–36.5 indicating mild to moderate autism, and scores ≥37 indicating severe autism. The tool has demonstrated good inter-rater reliability (ICC >0.80) and internal consistency in paediatric populations.

Statistical analysis was performed using SPSS software (version 27). Descriptive statistics were used to summarize demographic characteristics. Categorical variables were expressed as frequencies and percentages, while continuous variables were presented as means with standard deviations. Between-group comparisons were done using independent t-tests or Mann–Whitney U tests based on data distribution. Within-group pre- and post-

intervention changes in CARS-II scores were assessed using paired t-tests or Wilcoxon signed-rank tests. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The baseline characteristics of the participants in both the Quatre Folic (QF) and Standard Treatment (ST) groups were comparable, as summarized in Table 1. The mean age of children in the QF group was 3.51 ± 0.70 years, while in the ST group it was 3.37 ± 0.47 years (p = 0.525). Among the QF group, 33.3% were male and 66.7% were female, whereas in the ST group, 20.0% were male and 80.0% were female (p = 0.409). Anthropometric assessments revealed that the mean height-for-age Z-score was −1.06 ± 0.81 in the QF group and −0.91 ± 0.68 in the ST group (p = 0.597). The mean weight-for-age Z-score was −1.05 ± 0.93 in the QF group compared to −0.55 ± 0.77 in the ST group (p = 0.120), while the mean weight-for-height Z-score was −0.78 ± 0.72 and −0.98 ± 0.57 in the QF and ST groups, respectively (p = 0.399). The mean haemoglobin level was 11.57 ± 0.90 g/dL in the QF group and 11.13 ± 1.10 g/dL in the ST group (p = 0.247). The baseline CARS-II scores were also similar between the two groups, with a mean of 32.84 ± 1.36 in the QF group and 33.41 ± 1.28 in the ST group (p = 0.247). None of the differences were statistically significant, indicating well-balanced groups at baseline.

Table 1: Baseline characteristics of the study groups

		Group QF N = 15	**Group ST N = 15**	**P value**
		n (%)	**n (%)**	
Age (in years), Mean (SD)		3.51 (0.70)	3.37 (0.47)	0.525
Gender	Male	5 (33.3)	3 (20.0)	0.409
	Female	10 (66.7)	12 (80.0)	
Height-for-age Z-score, Mean (SD)		-1.06 (0.81)	-0.91 (0.68)	0.597
Weight-for-age Z-score, Mean (SD)		-1.05 (0.93)	-0.55 (0.77)	0.120
Weight-for-height Z-score, Mean (SD)		-0.78 (0.72)	-0.98 (0.57)	0.399
Haemoglobin (in g/dl), Mean (SD)		11.57 (0.90)	11.13 (1.10)	0.247
Baseline CARS-II score, Mean (SD)		32.84 (1.36)	33.41 (1.28)	0.247
*Statistically significant at p<0.05 SD, Standard deviation; CARS, Childhood Autism Rating Scale				

At the end of 12 weeks, significant improvements were observed in the QF group compared to the ST group across key outcome measures. The baseline mean CARS-II scores were comparable between the QF group (32.84 ± 1.36) and the ST group (33.41 ± 1.28), with no statistically significant difference (p = 0.247). However, after 12 weeks of intervention, the QF group showed a greater reduction in CARS-II scores (29.82 ± 1.89) compared to the ST group (32.36 ± 1.76), which was statistically significant (p = 0.001). The mean change in CARS-II score was 3.02 ± 1.17 in the QF group versus 1.05 ± 0.75 in the ST group, with a mean difference of −1.97 (95% CI: −2.76 to −1.17; p < 0.001), indicating a significantly greater reduction in autism severity among those who received Quatre Folic.

Regarding haemoglobin levels, the QF group had a baseline mean of 11.57 ± 0.90 g/dL, which increased to 11.77 ± 0.80 g/dL at 12 weeks. In contrast, the ST group showed minimal change (from 11.13 ± 1.10 to 11.12 ± 1.13 g/dL). The mean change in haemoglobin was significantly higher in the QF group (0.21 ± 0.02) compared to the ST group (−0.01 ± 0.02), with a mean difference of 0.22 (95% CI: 0.08 to 0.36; p = 0.003).

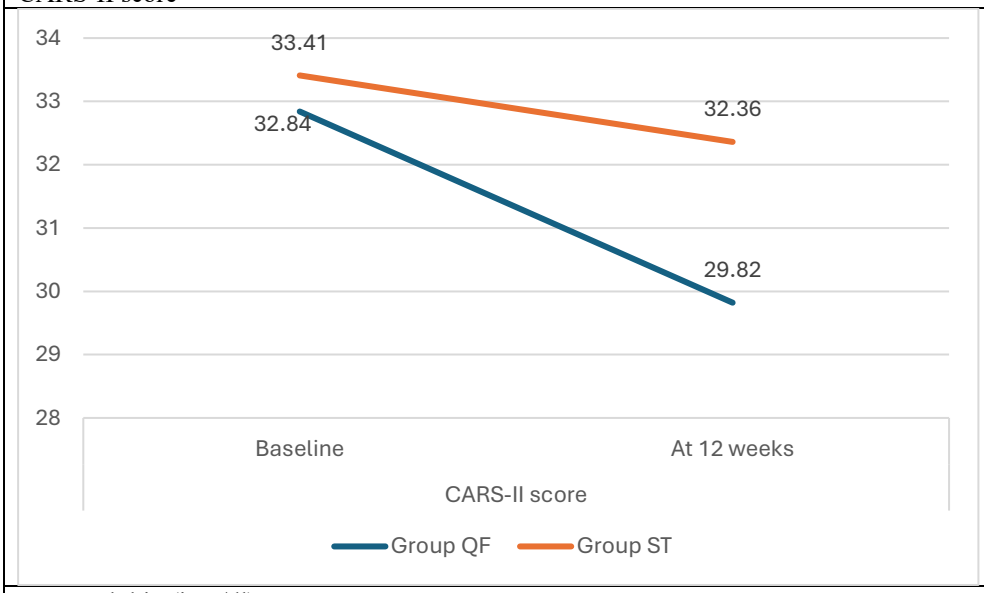
Weight-for-age Z-scores showed minimal changes in both groups. The QF group improved slightly from −1.05 ± 0.93 to −0.97 ± 0.94, while the ST group remained relatively stable (−0.55 ± 0.77 to −0.57 ± 0.79). Although the mean change in Z-score was greater in the QF group (0.08 ± 0.01) compared to the ST group (−0.02 ± 0.01), the difference was not statistically significant, with a mean difference of 0.10 (95% CI: −0.04 to 0.25; p = 0.182). Overall, the results demonstrate that Quatre Folic supplementation led to significant improvements in both autism symptom severity and haemoglobin levels.

Table 2: Comparison of study groups by CARS-II score, haemoglobin and weight-for-age Z-scores

		Group QF N = 15	Group ST N = 15	MD (95% CI)	P value
		Mean (SD)	Mean (SD)		
CARS-II score	Baseline	32.84 (1.36)	33.41 (1.28)	_	0.247
	At 12 weeks	29.82 (1.89)	32.36 (1.76)	_	0.001*
Change in CARS-II score		3.02 (1.17)	1.05 (0.75)	-1.97 (-2.76 to -1.17)	<0.001*
Haemoglobin (in g/dl)	Baseline	11.57 (0.90)	11.13 (1.10)	_	0.247
	At 12 weeks	11.77 (0.80)	11.12 (1.13)	_	0.088
Change in haemoglobin		0.21 (0.02)	-0.01 (0.02)	0.22 (0.08 to 0.36)	0.003*
Weight-for-age Z-score	Baseline	-1.05 (0.93)	-0.55 (0.77)		0.120
	At 12 weeks	-0.97 (0.94)	-0.57 (0.79)		0.240
Change in weight-for-age Z-score		0.08 (0.01)	-0.02 (0.01)	0.10 (-0.04 to 0.25)	0.182

*Statistically significant at p<0.05
SD, Standard deviation; CARS, Childhood Autism Rating Scale

CARS-II score



Haemoglobin (in g/dl)

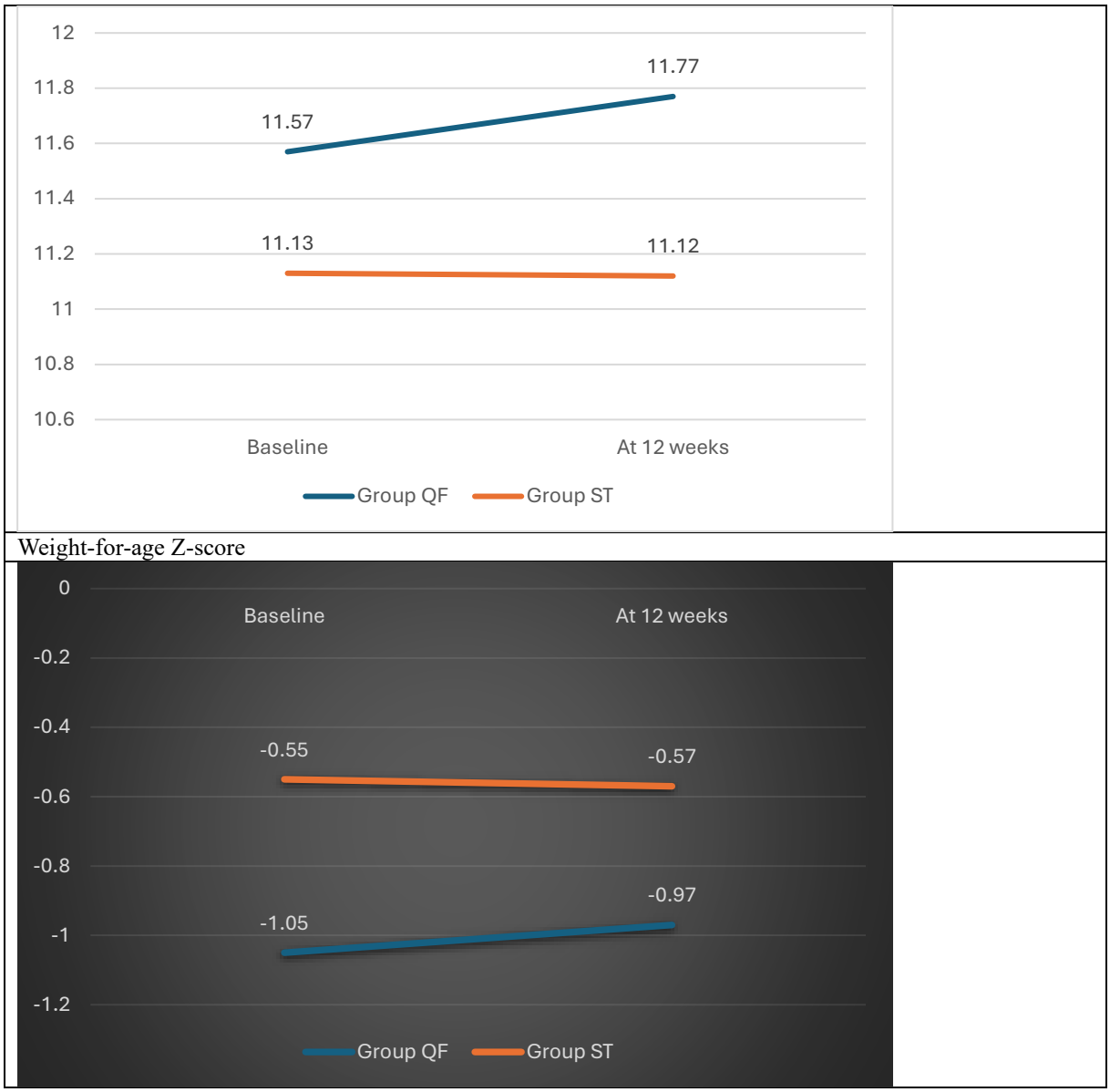


Figure 1: Comparison of study groups by CARS-II score, haemoglobin and weight-for-age Z-scores

DISCUSSION

The present double-blind, randomized controlled trial investigated the efficacy of Quatre Folic (active L-methylfolate) supplementation in reducing the severity of autistic symptoms in children under five years of age, using the CARS-II scale as the primary outcome measure. The findings demonstrated that children receiving Quatre Folic exhibited a significantly greater reduction in autism symptom severity and a notable improvement in haemoglobin levels compared to those receiving standard treatment alone. At baseline, the demographic, anthropometric, and clinical characteristics—including age, gender distribution, nutritional status (Z-scores), haemoglobin levels, and CARS-II scores—were comparable between the two groups, ensuring that the observed post-intervention effects were likely attributable to the supplementation rather than confounding variables. Following 12 weeks of intervention, the QF group showed a statistically significant reduction in CARS-II scores (mean change: 3.02 ± 1.17) compared to the ST group (mean change: 1.05 ± 0.75; $p < 0.001$). This corresponds to a moderate to large effect size, suggesting a clinically meaningful impact of L-methylfolate on core autistic features. The observed improvements may be explained by the role of folate in one-carbon metabolism,(13) which is essential for DNA methylation, neurotransmitter synthesis, and myelination—all critical for neurodevelopmental functioning.(14) Children with ASD have been found to exhibit folate pathway

abnormalities, including impaired transport of folate across the blood-brain barrier due to autoantibodies against folate receptor alpha (FRα).(15, 16) L-methylfolate, the bioactive form of folate used in this study, bypasses several metabolic steps and is readily available to cross the blood-brain barrier, which may explain its therapeutic efficacy.(17)

Similar to the present study, prior clinical trials have also demonstrated the benefit of folate-based interventions in ASD. A randomized trial by Frye et al.(12) (2018) showed that high dose folinic acid improved verbal communication and core autism symptoms in children with ASD, particularly those with FRα autoantibodies. While that study used folinic acid, the active metabolite used in our trial, Quatre Folic (L-5-methyltetrahydrofolate), offers enhanced bioavailability and avoids the need for enzymatic conversion by methylenetetrahydrofolate reductase (MTHFR)—a pathway often compromised in children with ASD.(18) Therefore, the present findings are consistent with and extend existing evidence on the therapeutic role of folate derivatives in autism management.

In addition to improvements in CARS-II scores, our study noted a modest but statistically significant increase in haemoglobin levels in the QF group (mean change: 0.21 g/dL; $p = 0.003$), while levels in the ST group remained unchanged. This suggests that Quatre Folic may also contribute to correcting subclinical nutritional deficiencies, potentially via enhanced erythropoiesis mediated by folate's role in DNA synthesis and red blood cell maturation.(19) Although the baseline haemoglobin levels were within normal range, the observed improvement reinforces the broader systemic benefits of optimized folate metabolism in paediatric populations. Changes in nutritional status, as reflected by weight-for-age Z-scores, were minimal and not statistically significant. This is not unexpected given the relatively short duration of the study and the primary neurological focus of the intervention. Long-term studies may be required to detect anthropometric improvements stemming from enhanced feeding behaviour or gastrointestinal function, which are often impaired in ASD.(20)

Taken together, these findings suggest that Quatre Folic supplementation is a promising adjunctive treatment for reducing core symptoms of autism and improving biochemical markers such as haemoglobin in young children. Given its excellent safety profile and ease of administration, L-methylfolate may be considered in individualized treatment plans, particularly for children with suspected or confirmed folate pathway abnormalities.

The present study has several limitations that should be acknowledged. Firstly, the sample size may limit the generalizability of the findings and reduce the statistical power to detect smaller effect sizes in secondary outcomes such as anthropometric changes. The study was conducted at a single center, potentially introducing site-specific biases and limiting external validity. Additionally, the duration of the intervention was limited to 12 weeks, which, although sufficient to observe short-term changes in autism severity and haemoglobin levels, may not capture long-term effects or sustained improvements in developmental outcomes. The study also did not assess underlying biomarkers such as folate receptor alpha autoantibodies, MTHFR gene polymorphisms, or homocysteine levels, which could have provided mechanistic insights and helped identify responders to treatment. Furthermore, although the CARS-II is a validated tool for assessing autism severity, reliance solely on this scale may not fully capture broader cognitive, behavioural, or language-related improvements. Finally, adherence to supplementation and potential dietary or environmental influences were not monitored, which could have influenced the outcomes and introduced uncontrolled confounding factors.

CONCLUSION

In conclusion, this randomized controlled trial demonstrated that Quatre Folic supplementation significantly reduced the severity of autistic symptoms, as measured by the CARS-II scale, and led to a modest but meaningful improvement in haemoglobin levels in children under five years of age with mild to moderate autism. The intervention was well tolerated and produced a greater therapeutic benefit compared to standard treatment alone over a 12-week period. These findings suggest that targeted folate supplementation using bioactive L-methylfolate may serve as an effective adjunct in the early management of autism spectrum disorder.

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