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REGULAR ARTICLE

Sulforaphane as an adjunctive treatment for irritability in children with autism spectrum disorder: A randomized, double-blind, placebo-controlled clinical trial

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Aim: Irritability related to autism spectrum disorder (ASD) complicates the management of ASD patients at home and in clinical settings. In this randomized, double-blind, placebo-controlled clinical trial, we aimed to investigate the beneficial effects of adjuvant treatment with risperidone and sulforaphane in alleviating the irritability of children with ASD.

Methods: Sixty drug-free patients aged 4–12 years were randomly assigned to one of two groups receiving risperidone plus sulforaphane or placebo. Risperidone was started with a daily dose of 0.25 mg in patients weighing <20 kg and 0.5 mg in those weighing ≥20 kg and increased stepwise to reach a maximum of 1 mg (<20 kg), 2.5 mg (20–45 kg), and 3.5 mg (>45 kg). Sulforaphane was administered at a daily dose of 50 μmol (≤45 kg) or 100 μmol (>45 kg). The participants were assessed with the Aberrant Behavior Checklist – Community Edition at baseline and at Weeks 5 and 10.

Results: Compared to the placebo group, ASD patients in the sulforaphane group showed greater improvements in Irritability score (primary outcome measure; $P = 0.001$) and Hyperactivity/Noncompliance score (secondary outcome measure; $P = 0.015$), and significant Time × Treatment effect for Irritability ($P = 0.007$) and Hyperactivity/Noncompliance ($P = 0.008$). However, no difference was seen in improvements in the other secondary measures: Lethargy/Social Interaction score, Stereotypic Behavior score, Inappropriate Speech score, and frequency of adverse events.

Conclusion: Our results support the safety and efficacy of sulforaphane as an adjuvant to risperidone for improvement of irritability and hyperactivity symptoms in children with ASD.

Keywords: *autism spectrum disorder, irritability, oxidative stress, risperidone, sulforaphane.*

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Autism spectrum disorder (ASD) is a heterogeneous group of neurodevelopmental disorders starting in early childhood. ASD is estimated to have a global prevalence of approximately 1% and affects one in 68 children aged younger than 8 years in the USA.[1, 2] ASD is primarily characterized by restricted interest, impaired social interactions, disrupted communications, and stereotypic behavior.[3] In addition to these core symptoms, ASD patients experience other associated symptoms, including cognitive dysfunction, anxiety, and irritability. [4–6]'Irritability' is defined as outbursts of vocal or motor fury and aggression. Recently, irritability has gained considerable attention as it leads to self-injury and complicates the adjustment of autistic patients both at home and in clinical environments.[7] However, there is no ideal pharmacological treatment for irritability in patients with ASD.

Although the etiology of ASD is not fully understood, multiple genetic, epigenetic, and environmental factors seem to play a role in disease pathogenesis.[3] A growing body of evidence shows a significant role for oxidative stress, inflammation, and mitochondrial dysfunction in the development of autism.[8, 9] Higher levels of oxidative biomarkers, including malondialdehyde, isoprostane, and nitric oxide, have been found in autistic patients.[10] Sulfur compounds are antioxidants that play a substantial role in detoxification.[11] Together with the increased oxidative stress in autistic patients, Adams *et al.*[12] found lower levels of plasma sulfate and decreased capacity for sulfation in these patients, and this may play an etiological role in the development of ASD. Furthermore, both central and peripheral immune systems are impaired in patients with ASD. Aberrant activation of microglia might lead to disruption of neural functions and, thus, contribute to the underlying pathogenesis of ASD.[13] Recent studies of ASD have also shown that the levels of peripheral immune cells, such as Foxp3+ T regulatory and Th1, Th2, and Th17 T helper cells, are disturbed in favor of excessive inflammation.[14, 15]

To date, none of the approved pharmacological treatments has led to an optimal improvement in associated symptoms of ASD, particularly irritability. Currently, the US Food and Drug Administration has only approved two atypical antipsychotics – risperidone (for patients older than 5 years) and aripiprazole (for patients older than 6 years) – for treatment of ASD. Although these medications may moderately decrease irritability and aggression,[3, 16, 17] they can result in serious adverse effects, including increased risk for diabetes and

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Individual participant data will be shared upon request.
This trial was registered with the Iranian Registry of Clinical Trials (www.irct.ir; No IRCT20090117001556N107).

obesity, extreme tiredness, gynecomastia, and extrapyramidal symptoms (i.e., dyskinesia).[16, 17]

Sulforaphane, found in *Brassica* vegetables, is an isothiocyanate produced from glucoraphanin by the myrosinase enzyme, which was initially known for its anti-carcinogenic properties.[18] Sulforaphane is an indirect antioxidant and anti-inflammatory compound. It enhances the activity of cytoprotective enzymes and plays a substantial role in detoxification and elimination of free radicals by several mechanisms. Low toxicity is the primary merit of this supplement.[19] Sulforaphane has been shown to mitigate many of the same underlying molecular abnormalities associated with ASD, such as decreased antioxidant capacity, mitochondrial dysfunction, increased lipid peroxidation, and neuroinflammation.[18] The protective effect of sulforaphane has been documented in one recent study on a murine model of autism. Nadeem *et al*.[20] demonstrated that sulforaphane ameliorates autism-like behaviors through suppression of Th17-related signaling and its antioxidant properties in the peripheries and brains of BTBR T + Itpr3tf/J (BTBR) mice. A few clinical studies have investigated the effects of sulforaphane on autistic patients. In a randomized, double-blind clinical trial, Singh *et al*.[21] investigated the efficacy of sulforaphane in a group of young adult autistic patients. They reported a significantly greater behavioral improvement in patients taking sulforaphane compared to the placebo group following 18 weeks of treatment. In a follow-up case series, the same investigators found that among patients who were receiving sulforaphane in the trial, almost 56% of responders had continued taking sulforaphane for 3 years.[16] Another recent open-label study also found significant improvement in the behavior and social interactions of patients who received adjuvant sulforaphane therapy.[22] However, both studies had small sample sizes, with fewer than 45 participants.

Given the evident role of neuroinflammation and oxidative stress in ASD, we hypothesized that the anti-inflammatory and antioxidant properties of sulforaphane might lead to an improvement in associated symptoms of ASD and, in particular, irritability. In this randomized, double-blind, placebo-controlled clinical trial, we investigated the effects of sulforaphane as an adjuvant therapy with risperidone on irritability and other associated symptoms of ASD.

Methods

Trial design and setting

This was a randomized, double-blind, placebo-controlled clinical trial conducted at the autism clinic in the children's outpatient clinic of Roozbeh Hospital (Tehran University of Medical Sciences, Tehran, Iran) from April 2018 to November 2019 on two parallel groups: sulforaphane and placebo. The protocol of the trial was in concordance with the ethical principles stated in the Declaration of Helsinki.[23] The institutional review board/ethics committee of Tehran University of Medical Sciences approved the protocol of the trial (Code No. IR.TUMS.VCR.REC.1396.4660). Written informed consent was obtained from patients' parents before enrollment in the trial. The parents or guardians of the children were educated about the possible side-effects of the medications. We also provided the parents or guardians with a helpline in case of any enquiries in this regard. The protocol of this trial was registered at the Iranian Registry of Clinical Trials (IRCT: http://www.irct.ir) with registration number IRCT20090117001556N107. Clinical examinations of participants were conducted on three separate events: baseline/screening, Week 5, and Week 10.

Participants

Enrolled patients were Iranian children aged 4–12 years from both male and female outpatients referred to our clinic from different parts of Iran with probable autistic signs and symptoms and meeting the DSM-5 criteria.[24] Two expert pediatric psychiatrists confirmed the diagnosis of ASD based on the children's behavioral examination and semi-structured interviews with the caregivers (Autism Diagnostic Interview – Revised).[25] We included patients manifesting irritability symptoms with at least moderate severity. 'Moderate severity' was defined as scores ≥15 on the Aberrant Behavior Checklist – Community Edition (ABC-C).[26] Children whose presentation at baseline was not severe enough to be considered for risperidone treatment were excluded. Also, patients were ineligible to enter the trial if they had: (i) concurrent prominent psychiatric disorder; (ii) preexisting medical conditions (in particular epileptic disorders and febrile seizures); (iii) intellectual disability (IQ < 70); (iv) history of drug or alcohol abuse; (v) history of tardive dyskinesia; or (vi) history of taking antipsychotic medication within 6 months prior to enrollment. To avoid asking patients to stop taking any medications before entry and to follow the ethical guidelines, only patients who had been drug-free for at least 6 months were included. Additionally, the cell blood count, basic biochemistry and electrolytes, and liver and kidney parameters of included participants were assessed as screening tests at baseline. The screening results were in the normal range for all participants.

Interventions

Participants in both groups received risperidone in a similar manner. The starting daily dose of risperidone was 0.25 mg in children weighing <20 kg and 0.5 mg in children weighing ≥20 kg. The dosage was increased stepwise by 0.5 mg weekly up to a maximum dose of 1 mg for children weighing <20 kg, 2.5 mg for those weighing 20–45 kg, and 3.5 mg for those weighing >45 kg. Sulforaphane (1-isothiocyanato-4-methylsulfinylbutane; ACER, Tehran, Iran) was prescribed at 50 µmol and 100 µmol (approximately 10 mg and 20 mg) per day for patients weighing <45 kg and 45–90 kg, respectively. The placebo group received placebo capsules. Risperidone and sulforaphane/placebo treatments were initiated simultaneously. No other concomitant intervention or medication was permitted. Adherence to treatment was evaluated by checking with parents and capsule-counting.

Outcomes and tools

The design, administration, and scoring of ABC-C is fully described elsewhere.[27, 28] The ABC-C is a valid and reliable tool for assessment of the severity of behavioral abnormalities seen in developmental disorders of the nervous system. It is a 58-item questionnaire designed to investigate five domains of behavioral impairments, including Lethargy/Social Withdrawal, Stereotypic Behavior, and Inappropriate Speech as the core symptoms of ASD and Irritability and Hyperactivity/Noncompliance as the associated symptoms of ASD.[29–31] The primary outcome measure of this trial was the mean change in the score for the Irritability subscale from baseline/screening to the study endpoint. The secondary outcome measures consisted of mean changes in scores for the ABC-C Lethargy/Social Withdrawal, Stereotypic Behavior, Inappropriate Speech, and Hyperactivity/Noncompliance subscales and the occurrence rate of adverse events. After education by investigators, the parents completed the ABC-C.

Adverse events

The adverse events were carefully monitored by a pediatric psychiatrist at baseline and at each follow-up visit (Weeks 5 and 10). Adverse events were recorded using a checklist containing 25 possible side-effects of the medications.[32–34] The potential extrapyramidal symptoms, including parkinsonism, akathisia, dystonia, and tardive dyskinesia, were monitored and recorded using the Extrapyramidal Symptom Rating Scale at baseline, and Weeks 5 and 10.[35, 36] In addition, 1 week after the beginning of the study, a phone call was made to the participants to record any adverse effects. Patients were also provided with a 24-h medical helpline phone number for medical advice in case they experienced any adverse effect.

Sample size

The initial sample size of 50 was calculated considering the following assumptions: (i) a mean difference of 3 between the two groups on the ABC-C Irritability subscale with a standard deviation of 3; (ii) a

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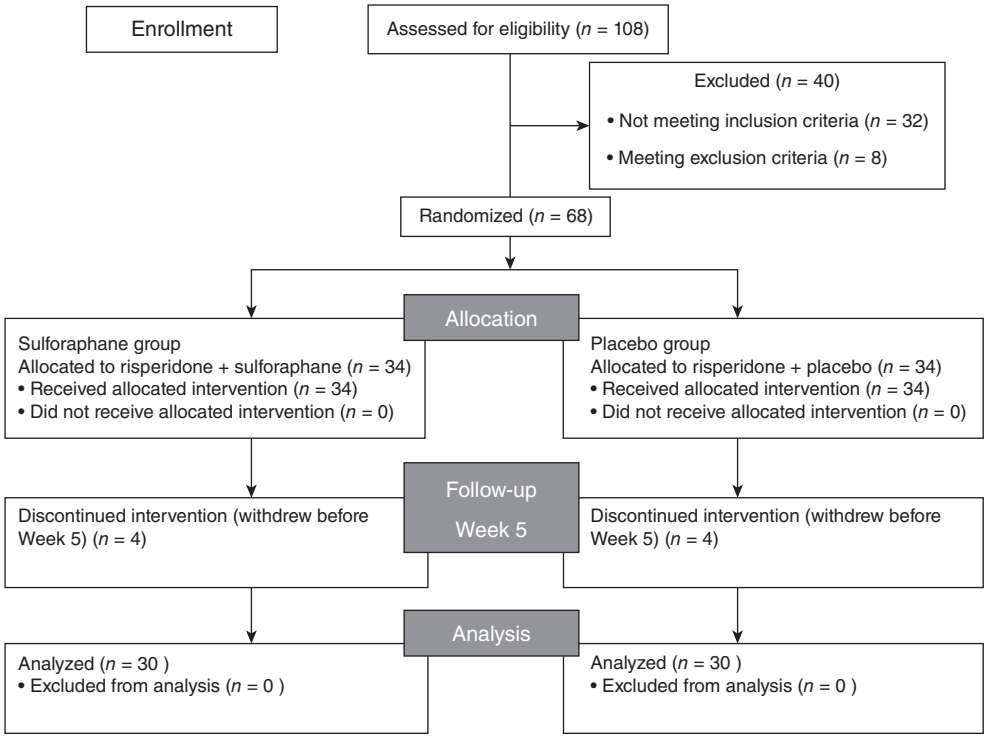

Fig.1 Flow diagram of the study.

Table 1. Baseline demographic and clinical characteristics of the patients in the two study groups

	Sulforaphane group (n = 30)	Placebo group (n = 30)	P-value
Age, mean (SD), years	6.87 (2.06)	7.67 (2.35)	0.17††
Male (%)	19 (63.3%)	21 (70.0%)	0.78‡‡
Weight, mean (SD), kg	23.40 (8.45)	26.13 (8.13)	0.21††
Irritability score, mean (SD)	22.50 (4.89)	21.30 (6.13)	0.40††
Lethargy/Social Withdrawal score, mean (SD)	20.90 (6.10)	19.97 (8.25)	0.62††
Stereotypic Behavior score, mean (SD)	12.10 (4.26)	11.40 (5.34)	0.58††
Hyperactivity/Noncompliance score, mean (SD)	28.47 (5.24)	26.67 (8.22)	0.32††
Inappropriate Speech score, mean (SD)	6.07 (2.69)	5.37 (3.19)	0.36††

P-value of <0.05 was considered statistically significant.
†Student's t-test.
‡χ^2-test.

power of 80%; and (iii) two-sided significance level of 5%. The final sample size was increased to 60 by giving an attrition rate of 20%. Considering a 1:1 enrollment ratio for the sample size, the number of required patients in each arm was 30 participants.

Randomization, allocation, concealment, and blinding

Each patient was assigned to a specific random code. The primary investigator of the study, who was not involved in the diagnosis and follow-up, conducted the randomization and allocation of the treatment groups using block randomization (with blocks of size 4). The assignments were kept in confidential and sealed opaque envelopes and were unveiled at the study end-point for statistical analysis. Randomizations, drug administration, rating, data entry, and statistical analysis were implemented by separate individuals. Placebo capsules were identical to sulforaphane based on shape, size, color, and taste.

Statistical analysis

All statistical analyses were carried out using SPSS Version 20 (IBM, Armonk, NY, USA). The sulforaphane and placebo groups were compared based on primary and secondary outcome measures of the study. Continuous variables are displayed as mean ± SD, and categorical variables are presented as frequencies with percentages. The general linear model (GLM) repeated-measures analysis was conducted to investigate the time, treatment, and Time × Treatment effects. The between-subjects factor was derived from the two treatment groups, and within-subject factors were the five ABC-C subscale scores. Greenhouse–Geisser correction for degrees of freedom was reported if Mauchly's test of sphericity was significant. Independent sample t-tests were used to compare the continuous variables between the two groups. The χ^2-test was used to compare sex and Fisher's exact test was used to compare the incidence of adverse effects between the two groups. To assess the difference in the outcome of the two groups, we calculated the mean difference in change score and respective

Table 2. Comparison of ABC-C scores and score changes between the two study groups

Clinical scores		Risperidone + sulforaphane group (n = 30), mean (SEM)	Risperidone + placebo group (n = 30), mean (SEM)	Mean difference (95%CI)	P-value
Irritability	Baseline	22.50 (0.89)	21.30 (1.12)	1.20 (−1.66 to 4.06)	0.405
	Week 5	17.57 (1.80)	17.50 (1.26)	0.07 (−4.33 to 4.47)	0.974
	Week 10	12.23 (0.70)	14.20 (1.04)	−1.97 (−4.49 to 0.55)	0.123
	Change from baseline to Week 5	−4.93 (0.93)	−3.80 (0.53)	1.13 (−1.03 to 3.29)	0.298
	Change from baseline to Week 10	−10.27 (0.78)	−7.10 (0.52)	3.17 (1.27 to 5.06)	**0.001**
Lethargy/Social Withdrawal	Baseline	20.90 (1.11)	19.97 (1.50)	0.93 (−2.82 to 4.68)	0.621
	Week 5	18.33 (0.98)	18.20 (1.61)	0.13 (−3.65 to 3.91)	0.945
	Week 10	17.40 (1.02)	16.57 (1.38)	0.83 (−2.60 to 4.26)	0.630
	Change from baseline to Week 5	−2.57 (0.70)	−1.77 (0.43)	0.80 (−0.84 to 2.44)	0.334
	Change from baseline to Week 10	−3.50 (0.87)	−3.40 (0.63)	0.10 (−2.05 to 2.25)	0.926
Stereotypic Behavior	Baseline	12.10 (0.77)	11.40 (0.97)	0.70 (−1.79 to 3.19)	0.576
	Week 5	9.97 (0.80)	10.17 (0.98)	−0.20 (−2.74 to 2.34)	0.875
	Week 10	9.23 (0.84)	9.43 (0.96)	−0.20 (−2.75 to 2.35)	0.876
	Change from baseline to Week 5	−2.13 (0.45)	−1.23 (0.31)	0.90 (0.20 to 2.00)	0.108
	Change from baseline to Week 10	−2.87 (0.60)	−1.97 (0.45)	0.90 (−0.60 to 2.40)	0.236
Hyperactivity/ Noncompliance	Baseline	28.47 (0.95)	26.67 (1.50)	1.8 (−1.76 to 5.36)	0.316
	Week 5	24.50 (1.07)	23.80 (1.58)	0.70 (−3.13 to 4.53)	0.716
	Week 10	20.77 (1.35)	22.43 (1.60)	−1.66 (−5.86 to 2.54)	0.432
	Change from baseline to Week 5	−3.97 (0.51)	−2.87 (0.69)	1.10 (−0.63 to 2.83)	0.208
	Change from baseline to Week 10	−7.70 (1.06)	−4.23 (0.89)	3.47 (0.68 to 6.25)	**0.015**
Inappropriate Speech	Baseline	6.07 (0.49)	5.37 (0.58)	0.70 (−2.66 to 4.06)	0.678
	Week 5	5.60 (0.48)	5.27 (0.57)	0.33 (−1.16 to 1.82)	0.659
	Week 10	5.10 (0.45)	4.90 (0.56)	0.20 (−1.25 to 1.65)	0.783
	Change from baseline to Week 5	−0.47 (0.20)	−0.10 (0.08)	0.37 (−0.08 to 0.81)	0.109
	Change from baseline to Week 10	−0.97 (0.23)	−0.47 (0.25)	0.50 (−0.19 to 1.19)	0.154

P-value of <0.05 was considered statistically significant (shown in bold).
ABC-C, Aberrant Behavior Checklist – Community Edition; CI, confidence interval; SEM, standard error.

confidence intervals (95%CI) between baseline and Week 5 and between baseline and Week 10. Independent sample t-test was used to compare mean changes in subscale scores (between baseline and each point at follow-up evaluation) between the two groups. A P-value of <0.05 was considered as statistically significant.

Results

Participants

As detailed in Figure 1, 108 potential cases aged 4–12 years with a diagnosis of ASD were screened for study eligibility; of these, 32 patients did not meet the inclusion criteria, and eight patients met the exclusion criteria. Eight patients (four patients from each treatment group) dropped out prior to the first post-baseline visit due to reasons including consent withdrawal and using other medications. Therefore, 60 patients were enrolled in the study and randomized into treatment groups with an equal allocation ratio of 1:1: (i) risperidone plus sulforaphane, and (ii) risperidone plus placebo.

Baseline data and clinical characteristics

Baseline clinical characteristics of study participants and baseline ABC-C subscale scores are detailed in Table 1. Patients in the sulforaphane and placebo groups were comparable based on age, sex, and baseline bodyweight. There was no significant difference between the two trial groups based on baseline ABC-C subscale scores.

Outcomes

The ABC-C subscale scores of the sulforaphane and placebo groups at baseline, Week 5, and Week 10, along with changes in scores at Weeks 5 and 10 from baseline are demonstrated in Table 2. The trial groups were comparable based on all five ABC-C subscales at baseline and the two post-baseline visits. Even so, both the sulforaphane and placebo groups showed significant improvements in all subscale scores of the ABC-C from baseline to Week 10 (study end-point; effect of time P-value < 0.001 for all).

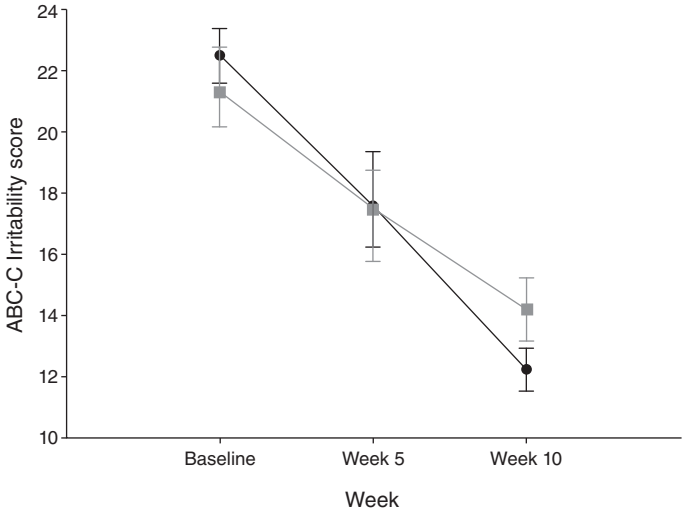

Fig.2 Comparison of Aberrant Behavior Checklist – Community Edition (ABC-C) Irritability score (mean [standard error]) between the (—●—) sulforaphane group and the (—■—) placebo group.

Primary outcome measure

Although there was no significant between-group difference based on change in Irritability score from baseline to Week 5 (*P*-value = 0.298), patients in the sulforaphane group showed significantly larger changes in Irritability scores from baseline to the study end-point (*P*-value = 0.001; Table 2 and Fig. 2). GLM repeated-measures analysis showed significant Time × Treatment interaction effect on the ABC-C Irritability subscale score ($F = 5.12$; d.f. = 2; *P*-value = 0.007).

Secondary outcome measures

No significant between-group difference was observed in changes to scores for ABC-C subscales Lethargy/Social Withdrawal (*P*-value = 0.334 and 0.926 for Weeks 5 and 10, respectively; Fig. 3), Stereotypic Behavior (*P*-value = 0.108 and 0.236 for Weeks 5 and 10, respectively; Fig. 3), and Inappropriate Speech (*P*-value = 0.109 and 0.154 for Weeks 5 and 10, respectively; Fig. 3) from baseline to Weeks 5 or 10 (Table 2). GLM repeated-measures analysis demonstrated no significant effect for Time × Treatment interaction on the Lethargy/Social Withdrawal ($F = 2.85$; d.f. = 1.53; *P*-value = 0.61), Stereotypic Behavior ($F = 1.59$; d.f. = 1.33; *P*-value = 0.21), and Inappropriate Speech ($F = 1.66$; d.f. = 1.59; *P*-value = 0.19) subscale scores. Compared to the placebo group, patients in the sulforaphane group showed a larger reduction in the Hyperactivity/Noncompliance subscale score from baseline to study end-point (*P*-value = 0.015; Fig. 3). However, no such difference was found from baseline to Week 5 (Table 2). GLM repeated-measures analysis demonstrated significant effect for Time × Treatment interaction on this subscale score ($F = 5.03$; d.f. = 1.51; *P*-value = 0.008).

Adverse events

No severe adverse events were observed and, thus, no one was excluded for this reason. Eight side-effects were observed; however, no unpredicted manifestations were found. The most frequent side-effects were increased appetite (13.3%) and headache (13.3%) in the sulforaphane group and diarrhea (20%) in the placebo group (Table 3). No significant between-group difference was found in the frequency of side-effects between the two trial groups. Regarding extrapyramidal symptoms, there was no significant difference in Extrapyramidal Symptom Rating Scale scores at Weeks 5 and 10 between the two trial groups.

Discussion

Given the heterogeneity of the underlying mechanisms of ASD, no single pharmacological treatment has been found to result in complete improvement of ASD behavioral symptoms. Thus, designing new adjunctive treatment strategies for ASD manifestation is considered an inevitable necessity. In this 10-week randomized, double-blind, placebo-controlled clinical trial, we assessed the therapeutic effects of adjunctive sulforaphane in management of irritability and other behavioral symptoms in children with ASD who were drug-free at the outset. The primary outcome measure of the study was ABC-C Irritability subscale score, while other ABC-C subscales, including Lethargy/Social Withdrawal, Stereotypic Behavior, Hyperactivity/Noncompliance, and Inappropriate Speech, were the secondary outcome measures.

We found that adjunctive treatment with sulforaphane leads to significant improvement in Irritability and Hyperactivity/Noncompliance in children with ASD. However, it did not show a significant effect on other subscales of the ABC-C.

A review of other studies assessing the effects of sulforaphane in autistic patients revealed quite similar results with slightly different findings. In 2014, Singh *et al*.[21] performed a randomized, double-blind, placebo-controlled trial with 29 adult male patients, aged 13–27 years, assessing the beneficial effects of sulforaphane on behavioral disruption of ASD. They prescribed daily oral doses of 50–150 μmol sulforaphane for 18 weeks. They reported a more considerable improvement in the Lethargy/Social Withdrawal and Stereotypic Behavior subscales of the ABC-C in addition to the Irritability and Hyperactivity/Noncompliance subscales. Furthermore, their findings showed that the beneficial effects of sulforaphane on ASD behavioral symptoms are present both in the short-term (after 4 weeks) and long-term (after 10 and 18 weeks) periods. However, we did not observe any significant improvements in ABC-C subscales after 5 weeks of treatment with sulforaphane. Their better results may be due to the following main reasons: First, they included young adult ASD patients, while our participants were ASD children. Older patients may respond faster and better to the adjuvant treatment with risperidone and sulforaphane, which might be due to the different rate of drug metabolism in children and adult patients. Second, the duration of treatment to observe the peak effect may be longer than 10 weeks. Furthermore, in 2018, Bent *et al*.[22] conducted a 12-week open-label study on 15 autistic children. In contrast to our study, they prescribed glucoraphanin, the precursor of sulforaphane, in addition to a conversion enzyme based on weight-based dosing (~2.5 μmol glucoraphanin/0.453 kg). Sulforaphane was also shown to be effective in improvement of Social Responsiveness Scale scores. In this regard, Singh *et al*.[21] reported more significant improvement in scores for the Awareness, Communication, Motivation, and Mannerism subscales after 18 weeks, while Bent *et al*.,[22] who followed up patients for 3 months, found a significantly better outcome only in the Communication and Motivation subscales.

Even though the etiology of ASD is not well known, several dysfunctions are found to play a key role in its induction, one of which could be increased oxidative stress with elevated levels of oxidative biomarkers, such as malondialdehyde, isoprostane, and nitric oxide.[9, 10, 37, 38] The abnormal oxidative stress in ASD patients can be caused by either dysfunction in clearance or production of reactive oxygen species.[22] Moreover, a growing body of evidence supports increased levels of pro-inflammatory cytokines in autistic patients.[39–43] The level of increased cytokines correlates with the severity of the patient's behavioral impairment.[36] The other potential culprit behind ASD is mitochondrial dysfunction, such as reduced number or activity of mitochondrial complexes.[44] Sulforaphane can ameliorate symptoms of ASD by targeting these abnormalities. Its primary function is to induce the erythroid 2-related factor 2 (Nrf2)–antioxidant response element pathway, which plays a crucial role in the regulation of cytoprotective and antioxidant enzymes.[19] Activation of this pathway

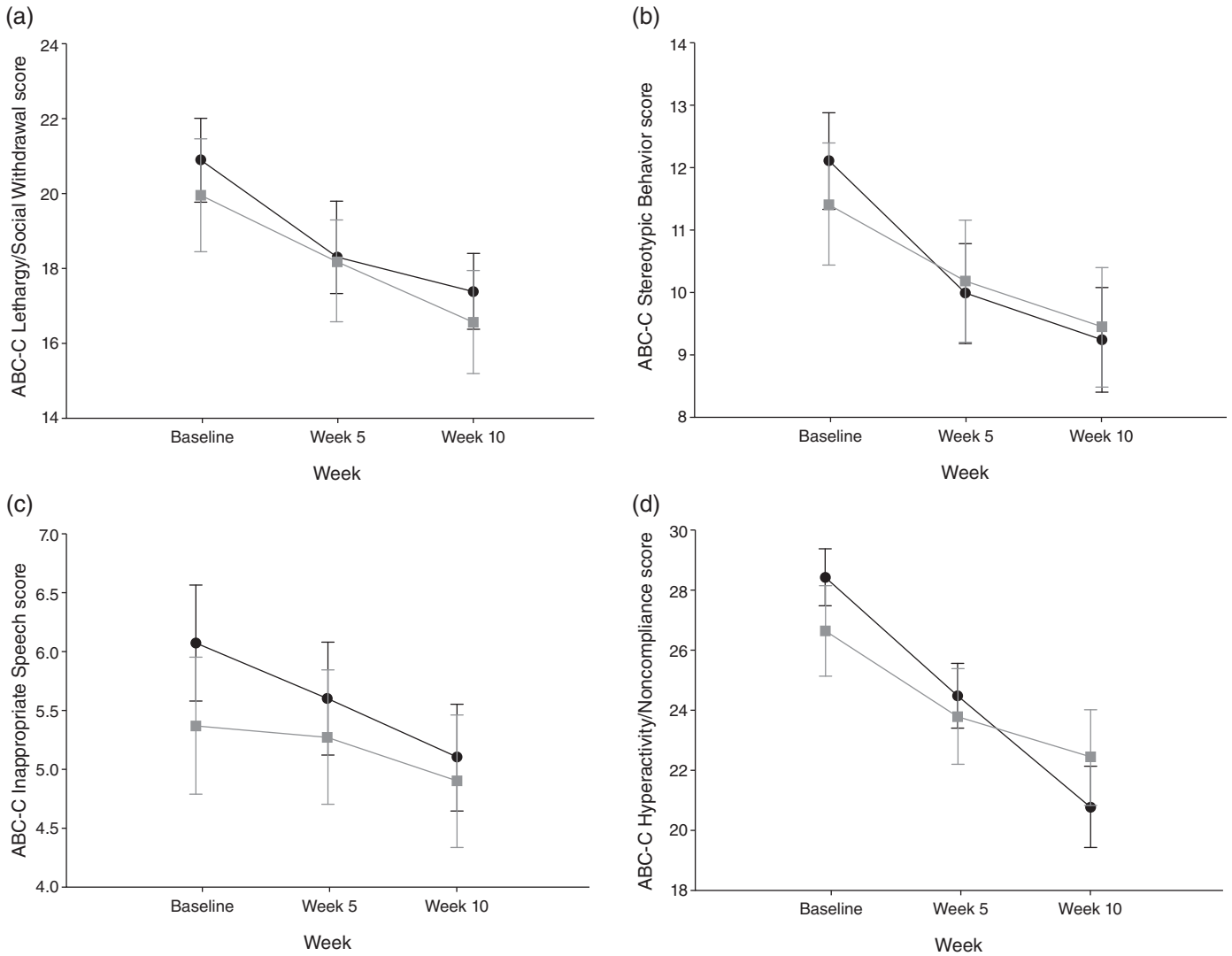

Fig.3 Comparison of Aberrant Behavior Checklist – Community Edition (ABC-C) (a) Lethargy/Social Withdrawal score, —●—, sulforaphane group; —■—, placebo group, (b) Stereotypic Behavior score, —●—, sulforaphane group; —■—, placebo group, (c) Inappropriate Speech score, —●—, sulforaphane group; —■—, placebo group and (d) Hyperactivity/Noncompliance score between the (—●—) sulforaphane group and the (—■—) placebo group. Data are shown as (mean [standard error]).

Table 3. Frequency of side-effects in the study population

Side-effect	Sulforaphane group (n = 30)	Placebo group (n = 30)	P-values
Dizziness, n (%)	1 (3.3%)	2 (6.7%)	1.000
Sedation, n (%)	2 (6.7%)	1 (3.3%)	1.000
Abdominal pain, n (%)	2 (6.7%)	3 (10.0%)	1.000
Increased appetite, n (%)	4 (13.3%)	3 (10.0%)	1.000
Headache, n (%)	4 (13.3%)	3 (10.0%)	1.000
Diarrhea, n (%)	1 (3.3%)	6 (20.0%)	0.103
Rashes, n (%)	1 (3.3%)	0 (0.0%)	1.000
Constipation, n (%)	0 (0.0%)	3 (10.0%)	0.237

P-value of <0.05 was considered statistically significant.
Fisher's exact test was used for comparison of all adverse events.

upregulates the production of NAD(P)H quinone oxidoreductase 1, glutathione peroxidase 1, heme oxygenase 1, and gamma-glutamylcysteine synthetase, which controls the production of glutathione.[45] Activations of the Nrf2-signaling pathway can also have anti-inflammatory effects and control neuroinflammation by several mechanisms, the first one of which is to promote polarization of microglia from M1 type (inflammatory) to M2 type (anti-inflammatory), lowering the levels of inflammatory mediators, such as the pro-inflammatory cytokines.[45, 46] It can also inhibit the activation of inflammatory mediators, such as the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) inflammatory response, and trigger production of anti-inflammatory compounds, like bilirubin and carbon monoxide.[44,47] Sulforaphane is also found to increase the release of glutathione, a major element in inhibiting the damage caused by free radicals by more than twofold in cultured astrocytes[48] and increasing the amount of this compound in human subjects' brains after 7 days.[49] This mechanism is supported by decreased urinary levels of metabolites of oxidative stress, such as γ-glutamylglutamine and methionine sulfone, in autistic patients taking sulforaphane.[22] The levels of these metabolites were negatively correlated with patient's behavior improvement.[22] The anti-oxidative and anti-inflammatory effects of sulforaphane make it an efficient treatment for ameliorating the underlying molecular abnormalities associated with ASD.[50]

In the BTBR T + Itpr3tf/J (BTBR) murine model of autism, Nadeem *et al.*[20] reported that sulforaphane mitigated autism-like behaviors, such as self-grooming/marble-burying behavior, and augmented social interaction in the three-chambered sociability test. They also demonstrated that these behavioral improvements were through suppressed Th17 immune activities (signal transducer and activator of transcription 3, RAR-related orphan receptor, interleukin-17 A, and interleukin-23 receptor expression in CD4 + T cells), inhibited oxidative stress parameters in neutrophils/cerebellum (NF-κB, inducible nitric oxide synthases, and lipid peroxides), and enhanced antioxidant enzymatic activity in neutrophils/cerebellum (superoxide dismutase, glutathione peroxidase, and glutathione reductase expression and activity). Furthermore, sulforaphane has been shown to alleviate behaviors associated with ASD, including anxiety and depression, in different animal models.[51, 52] These neuroprotective properties of sulforaphane are probably the reasons that we observed significant improvements in irritability and hyperactivity/noncompliance in children with ASD.

Our study is not without limitations. The duration of the trial was 10 weeks, which might not reveal the long-term benefits and side-effects of adjuvant treatment with sulforaphane. No measures of autism severity (e.g., social communication deficits and restrictive, repetitive behaviors) or clinical ratings have been reported in this study. In addition, six patients in this trial were aged between 4 and 5 years old and this does not comply with US Food and Drug Administration approval for use of risperidone in ASD. Finally, this was an adjunctive clinical trial, and sulforaphane monotherapy was avoided due to ethical considerations. Thus, our study did not assess the therapeutic effect of sulforaphane monotherapy on behavioral manifestations of ASD.

Irritability severely complicates the management of ASD patients both at home and in the clinic. The current ASD pharmacotherapy is not optimal for management of irritability. In this randomized, double-blind, placebo-controlled clinical trial, the potential therapeutic effects of sulforaphane on irritability and other behavioral impairments of patients with ASD were assessed. Our results support the safety and efficacy of sulforaphane as an adjuvant to risperidone in the treatment of autism. Sulforaphane can significantly increase the improvement of irritability and hyperactivity symptoms in autistic children. However, further studies are required to determine the long-term effect of this compound on other symptoms of autism.

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Disclosure statement

The authors declare no conflict of interest.

Author contributions

S.M. drafted the article, critically revised the manuscript for important intellectual content, and approved the final version. Z.A.-Y. developed the concept and design, collected the data, critically revised the manuscript for important intellectual content, and approved the final version. H.S.M. critically revised the manuscript for important intellectual content, prepared the figures and tables, and approved the final version. M.R.M. conducted statistical analysis and approved the final version. S.A. developed the concept and design, collected the data, supervised the project, critically revised the manuscript for important intellectual content, and approved the final version.

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