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Ajwa Dates Reduce Oxidative Stress in Egyptian Children with Autism: A Six-Month Randomized Controlled Trial

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Abstract

Background: Increased reactive oxygen species (ROS) and reduced antioxidant defenses contribute to the development of autism spectrum disorders (ASDs). Ajwa dates (Phoenix dactylifera) are recognized for their antioxidant properties, which may help neutralize free radicals and mitigate oxidative stress.

Aim: This randomized controlled trial aimed to evaluate the effects of Ajwa date consumption on oxidative stress markers in 131 Egyptian children with autism aged 3 to 12 years. The study compared changes in oxidant/antioxidant balance ratios between date-consuming and non-date-consuming groups. Additionally, it explored the relationship between autism severity and the effectiveness of date regimens in reducing oxidative stress, investigated long-term effects post-intervention, and assessed how baseline characteristics influence responses to date intake.

Method: Participants were randomly assigned to three groups: consuming three dates per day (47 children), five dates per day (42 children), or serving as a non-date control group (42 children). The intervention spanned 24 weeks, including a 12-week active consumption phase followed by a 12-week follow-up to assess long-term effects. Blood samples were collected at baseline, after 12 weeks of intervention, and 12 weeks post-intervention to measure oxidative stress markers, such as Malondialdehyde (MDA), glutathione peroxidase (GPx1), and superoxide dismutase (SOD).

Results: Ajwa date consumption significantly improved oxidative stress markers in children with autism, independent of dose. After 12 weeks, both date regimens led to significant reductions in MDA/SOD and MDA/GPx1 ratios ($p < 0.001$). Compared to the non-date group, 82% of children in the date groups showed improvement, versus 40.5% and 47.6% in the non-date group for the MDA/SOD and MDA/GPx1 ratios, respectively ($p < 0.01$). The improvements were particularly pronounced in children with moderate to severe autism. While oxidant/antioxidant balance slightly declined three months post-intervention, it remained significantly better than baseline levels.

Conclusion: The study demonstrates that Ajwa dates can effectively reduce oxidative stress markers in children with ASD, independent of dose. Residual benefits persisted after discontinuing Ajwa date intake, though less pronounced than those observed during the intervention period. The improvements were strongly associated with date intake, regardless of baseline characteristics, suggesting that Ajwa dates are a promising complementary treatment for managing oxidative stress in children with ASD.

Clinical Trial Registration: https://clinicaltrials.gov/ct2/show/NCT04261595, Identifiers: NCT04261595, Unique Protocol ID: 12060158

Keywords: Antioxidant Activity, Ajwa Dates, Autism Spectrum Disorder (ASD), Nutritional Intervention, Reactive oxygen species, MDA/SOD Ratio, MDA/GPx1 Ratio.

Introduction: Autism Spectrum Disorders (ASDs) are complex neurodevelopmental syndromes characterized by persistent deficits in social communication and restricted, repetitive behaviors. Globally, ASD prevalence is rising, and in Egypt, the estimated prevalence among children aged 1 to 12 years at high risk for ASD is approximately

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3.3% [1]. While many signalling pathways have been implicated in ASD, the precise etiology remains unclear, likely involving a multifactorial interaction of genetic predispositions and environmental influences, making treatment of ASD particularly challenging [2].

One of the critical pathophysiological mechanisms in ASD is the imbalance between reactive oxygen species (ROS) production and antioxidant defenses. Several studies have linked elevated ROS levels and decreased antioxidant capacity to the development and progression of ASD [3-7]. ROS, naturally produced during metabolic processes, are typically neutralized by the body's antioxidant defense system, maintaining a state of redox balance. When ROS production exceeds antioxidant defenses, oxidative stress occurs, leading to cellular damage and contributing to ASD pathogenesis [8].

The role of oxidative stress in ASD is further supported by numerous studies showing increased levels of oxidative stress markers in individuals with ASD. For instance, malondialdehyde (MDA), a byproduct of lipid peroxidation, is consistently elevated in children with ASD, indicating excessive oxidative damage to cell membranes [5, 6]. Additionally, antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx1), and catalase (CAT), which play key roles in neutralizing ROS, have been found to be altered in individuals with autism compared to healthy controls [5, 6].

Another emerging concept is the role of neuroinflammation in ASD, defined as an inflammatory response within the central nervous system (CNS) mediated by ROS. Neuroinflammation can impair mitochondrial function, further compromising the antioxidant defense system and exacerbating oxidative stress. This cascade of events leads to abnormalities in brain development, contributing to neurodevelopmental disorders such as ASD and ADHD [2, 9, 10]. Consequently, therapies targeting oxidative stress and neuroinflammation are considered promising strategies for managing ASD symptoms.

Dates (Phoenix dactylifera), which are rich in antioxidant compounds, could be included as a potential dietary intervention to combat oxidative stress in ASD. Dates are widely consumed across the world, particularly in the Middle East, and have been traditionally recognized for their health benefits in preventing and managing chronic diseases. The date fruit is a natural source of potent antioxidants, including phenolic compounds such as p-coumaric, ferulic, and sinapic acids, flavonoids, and procyanidins [11]. These compounds exhibit strong free radical scavenging activity due to their redox properties, helping to neutralize ROS and restore redox balance in cells [12].

Studies have shown that date consumption enhances the activity of antioxidant enzymes such as SOD and GPx1, while significantly reducing MDA levels [13]. Furthermore, dates have been demonstrated to reduce oxidative damage, inflammation, and apoptosis, making them a potential therapeutic option for conditions characterized by high oxidative stress [14] The molecular mechanisms underlying the antioxidant effects of dates include free radical quenching, inhibition of prooxidant enzymes like NADPH oxidase, and modulation of cellular signaling pathways involved in oxidative stress and inflammation [15]

In light of these findings, the present study aimed to evaluate the potential benefits of Ajwa date consumption in reducing oxidative stress markers in Egyptian children with ASD. Specifically, this study assessed the effect of date consumption on key oxidative stress markers, including MDA, SOD, and GPx1, and explored the relationship between autism severity and the effectiveness of date intake in reducing oxidative stress. Additionally, the study investigated the long-term effects of date consumption post-intervention. Finally, we examined how baseline characteristics may influence children's responses to the antioxidant effects of dates.

Subjects and Methods

Study Design: A randomized controlled clinical trial was conducted from June 2019 to June 2022. Simple randomization (1:1:1 ratio) was implemented by the clinical research coordinator after verifying inclusion and exclusion criteria. To minimize observer bias, all assessments and evaluations were performed in a blinded manner. Randomization was stratified by age, sex, and disease severity using unique identifiers, ensuring a balanced distribution of baseline characteristics across the groups.

Study Population: Participants were recruited from children with autism attending both the outpatient clinics of Children with Special Needs outpatient clinic, Developmental and Behavioural Pediatric clinic and Clinical Genetics Department clinic, at the Centre of Excellence for Medical Research, National Research Centre. The clinical diagnosis of autism spectrum disorder (ASD) was confirmed through the following psychometric assessments: the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision* (DSM-5) [16], and the *Childhood Autism Rating Scale* (CARS) [17]. The DSM-5 serves as the standard classification system used by health care professionals to assess and diagnose autism [18], while CARS is observational tool with high inter-rater reliability and validity [19]. Inclusion and exclusion criteria were verified by specialized pediatricians through detailed medical histories and comprehensive physical exams.

Inclusion Criteria:

- Children diagnosed with ASD, aged 3 to 12 years at study initiation.
- No history of allergic reactions to dates, which has been confirmed by oral food challenge test. The child ate slowly fragmented parts of one piece of Ajwa (10-15 gram), and was closely watched by an allergist for any allergic reaction.
- No major changes in medical treatment within two months prior to the study, with no plans for such changes during the trial period.
- Willingness to consume dates daily for three months as directed.

Exclusion Criteria:

- Children with other causes of mental sub-normality, congenital syndromes, or neurological conditions.
- Recent consumption (within the past four weeks) of probiotics, dates, or antibiotics/laxatives within the last six months.

- Major medical conditions, including cardiac, liver, endocrine, or renal diseases.
- History of seizure disorder or significant neurological deficits.
- When ASD was part of another condition such as Fragile X syndrome, tuberous sclerosis, or phenylketonuria.

Children with the above-mentioned disorders were excluded following medical history review and genetic assessments conducted by the professional clinical genetics team from the National Research Centre. This team was responsible for confirming any suspected genetic disorders with distinct physical features or with positive laboratory reports.

Sample Size: The target sample size for each treatment group was set at 32 participants, providing an 85% statistical power to detect significant differences between groups. This calculation was based on an expected improvement of 1.5 times or more resulting from date fruit consumption. A two-sided Z test with pooled variance was employed, with a target significance level of 0.05 and an error rate of 0.0587. To account for potential attrition, a 15% loss (n=5) was factored in, increasing the expected enrollment to 37 children per group. This figure was rounded to 40 participants per group, resulting in a total of 120 children with ASD across the three treatment regimens. The final sample size aimed to ensure at least 32 children remained in each group by the study's end [20, 21].

Allocation of participants in intervention and control groups

A total of 183 children diagnosed with autism were randomly assigned to one of three groups: two intervention groups (Ajwa dates group 1 and Ajwa dates group 2) and one control group (non-Ajwa dates group). The allocation of participants was designed to maintain the balance between groups regarding their gender and age. The mean age was 6.3± 2.2 years.

Parents and caregivers were provided with clear instructions on administering the date fruit regimen. Ajwa dates were consumed either during breakfast or as a snack between breakfast and lunch, with a requirement that no tea be consumed for at least one hour following the intake of the dates.

- **1st Regimen (Ajwa Dates Group 1):** Children diagnosed with autism in this group consumed three pieces of Ajwa dates (approximately 10-15 g each) daily for 12 weeks.
- **2nd Regimen (Ajwa Dates Group 2):** Children in this group, matched by age and sex to those in the first regimen, consumed five pieces of Ajwa dates (approximately 10-15 g each) daily for 12 weeks.
- **3rd Regimen (Non-Dates Group):** Children diagnosed with autism in this group, matched by age and sex to those in the intervention groups, did not consume dates. They maintained their regular diet and medications throughout the study. Unlike the intervention groups, this group was not restricted from taking antioxidants or vitamins if prescribed.

This trial represents a novel investigation into the effects of date fruit on autism. The minimum dosage of three dates was selected based on a 2019 study by Al Jaouni and colleagues [22], which demonstrated positive effects of Ajwa date consumption on infection rates, hospitalization, and survival outcomes in pediatric cancer patients. Furthermore, the choice to include five dates in the second regimen aligns with the recommendation from Prophet Mohammed (Peace Be upon Him), who stated, “If somebody takes five to seven Ajwa dates in the morning, neither magic nor poison will harm him that day." [23 & 24] This dose recommendation was applied to both children and adults, justifying its use in the study.

The 24-week duration of the intervention was chosen to allow sufficient time for both short-term (12 week duration) and long-term effects (additional 12 week duration) of date fruit consumption to manifest, without significantly affecting the participants' weight.

Intervention Type: Ajwa Dates Fruit (Phoenix dactylifera)

The Ajwa Dates (Phoenix dactylifera) used in this study were sourced from Al-Madinah, Saudi Arabia, known for its organic dates with exceptional nutritional and pharmacological properties [25 &26]

To justify the inclusion of Ajwa Dates in children with autism, the chemical composition of 15 pieces of Ajwa date flesh (approximately 100 g) was analysed at the Safe Food Unit of the National Research Centre (NRC) in Egypt. This analysis was conducted prior to the study to provide a solid base of data, ensuring sufficient evidence for testing the fruit in children with autism. The nutritive composition of Ajwa dates was previously documented by Metwally and colleagues [27] providing information on the daily recommended allowance of key nutrients for children aged 3-12 years.

Ajwa dates were found to be a high-energy food due to their rich sugar content, comprising 74.2% of their dry weight. Glucose and fructose were identified as the primary sugars, while sucrose was present in minor quantities. The amino acid profile of Ajwa date flesh revealed a high percentage of both essential and non-essential amino acids, with lysine (72 mg/100 g), valine (65 mg/100 g), and leucine (58 mg/100 g) as the major essential amino acids. The non-essential amino acids were dominated by aspartic acid and glutamic acid [27]. Amino acids play critical roles in protein synthesis, neurotransmitter production, and various metabolic processes. [28]

The analysis also highlighted significant levels of ascorbic acid (Vitamin C), which is essential for cellular function and numerous biochemical reactions [26]. Additionally, major phenolic compounds found in Ajwa dates were analyzed at NRC showing strong antioxidant properties with potential benefits for oxidative stress-related diseases and infectious conditions, as previously reported in the literature [29]

Study Visits, Safety, and Adverse Events

Participants attended five study visits. ***The baseline visit (Visit 1)*** involved an initial assessment of inclusion and exclusion criteria, parental consent, and a detailed medical history. This visit also collected demographic data, current medication or supplements, and pediatrician-confirmed inclusion criteria. Baseline dietary assessments, anthropometric measurements, and blood samples for oxidative stress markers (Malondialdehyde (MDA), glutathione peroxidase (GPx1), and superoxide dismutase (SOD)) were also taken. Participants were then randomly assigned to one of the three regimens.

Visits 2 and 3 (Follow-up Visits) occurred one and two months after the baseline visit. These visits monitored compliance, recorded any adverse or unusual events, and confirmed the continued intake of Ajwa dates for the intervention groups.

Visit 4 (First Evaluation) took place 12 weeks after the baseline visit. The same tests performed at Visit 1 were repeated, and blood samples were collected to assess the effects of Ajwa date consumption on oxidative stress markers.

Visit 5 (Second Evaluation) occurred 12 weeks after discontinuing the intervention. This final visit assessed the sustained effects of the intervention by repeating the tests from Visits 1 and 4. The study design and its phases are summarized in Figure 1.

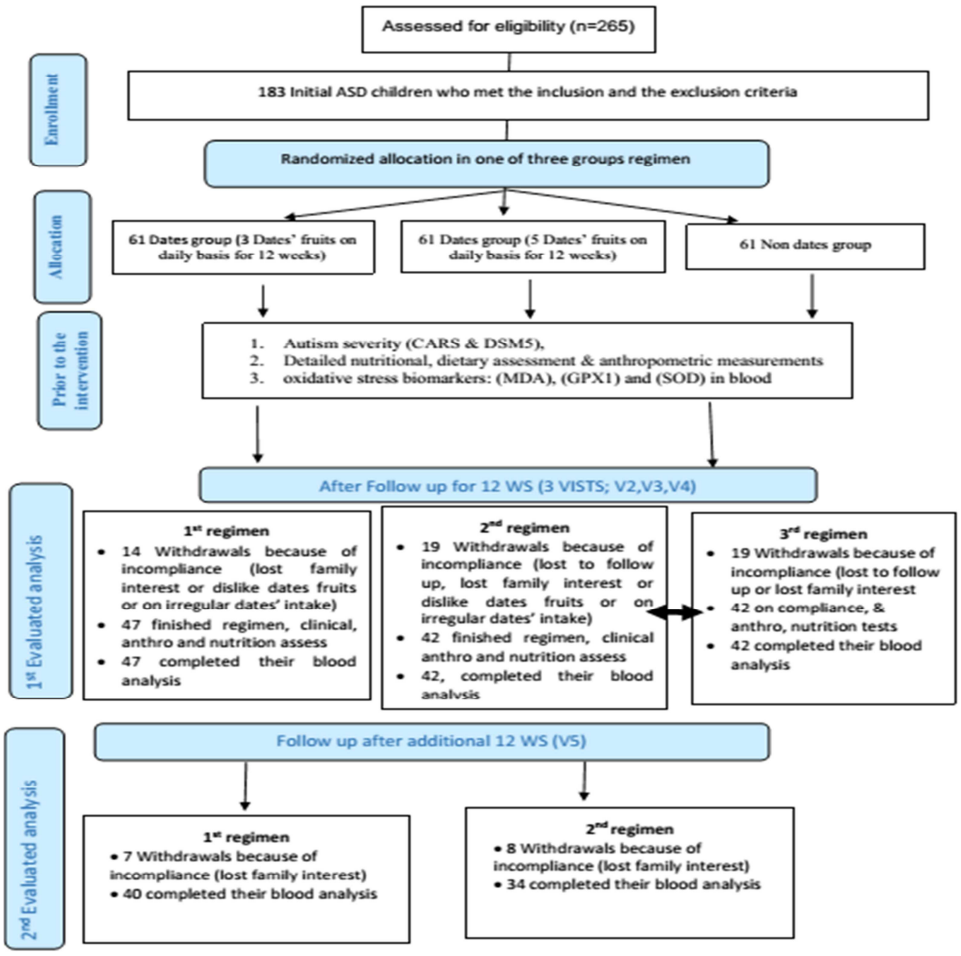

Figure (1): Flow Diagram for the study approach

Instruments for Assessment and Evaluation of Clinical and Laboratory Variables and Improvement Outcomes

Severity of ASD:

The two core symptoms of ASD—impaired social communication and the restricted or repetitive patterns of interests and behaviors—are currently used to determine the severity of the disorder. However, subjects with ASD frequently experience additional difficulties that have a significant impact on their daily lives, wellbeing, and need for support. These difficulties include intellectual disability, language delay, epilepsy, GIT symptoms, sleep disorders, ADHD, and anxiety disorders [30]. The severity levels of autism can be accurately assessed using a variety of standardized tools, including the Autism Rating Scale (CARS) [31], and the gold standard measures, the Autism Diagnostic Interview-Revised (ADI-R) [32] and the Autism Diagnostic Observation Schedule (ADOS) [33]. These tests are able to reliably assess the degree of severity of the core symptoms. However, they were not intended to assess the severity of autism in relation to the co-occurring conditions. They didn't provide a complete understanding of how much autism affects day-to-day functioning and the independency. A multifaceted approach is required to classify the functional impairment of ASD [34].

In the current study, authors preferred to depend on the DSM-5 classification. In which, the severity levels for the core symptoms of autism are obviously posited to correspond to three levels of functional impairment. Level 1, "Support needed"; Level 2 "Substantial support needed"; and Level 3, "Very substantial support needed." Thus, the DSM-5 attaches degrees of impairment and required support to the severity levels of specific behaviors [35].

Anthropometric Measurements:

Body weight (kg) and height (cm) were measured at the baseline of the study using a digital scale and a wall-mounted stadiometer, with subjects in minimal clothing and no shoes. Body Mass Index (BMI) was calculated by dividing body weight (kg) by height squared (m^2). All anthropometric measurements were converted to Z-scores specific to the child's age and gender using the World Health Organization (WHO) child growth standards (WHO Anthro). Obesity, defined as increased adiposity, served as a proxy for body fat. The classification for BMI Z-scores is as follows [36]: ≤1 SD: Non-obese, >1 SD: Overweight, >2 SD: Grade 1 obesity and >3 SD: Grade 2 obesity.

Improvement in BMI was deemed significant if there was a shift of at least one standard deviation in Z-score (e.g., from overweight to normal weight, or from grade 2 obesity to grade 1 obesity). BMI was assessed both before and after the trial, with comparisons made across the three treatment regimens.

Dietary Assessment:

A detailed dietary evaluation was conducted using the Diet Quality Index Assessment Questionnaire [37]. Improvement in dietary habits was considered significant if there was a transition from inevitable risk of malnutrition to possible risk, or from possible risk to no risk. A maintained status of "no risk" was also considered a positive outcome.

Serum Oxidant and Antioxidant Analysis:

Whole blood samples (5 ml) were collected via venipuncture in sterile conditions and the sera were separated and stored at -20°C for subsequent analysis. The following markers were measured using enzyme-linked immunosorbent assay (ELISA) kits from Elabscience (USA):

- Malondialdehyde (MDA) (Cat. No.: E-EL-0060) – marker of oxidative stress
- Glutathione Peroxidase (GPx1) (Cat. No.: E-EL-H5410) – antioxidant enzyme
- Superoxide Dismutase (SOD) (Cat. No.: E-EL-H1113) – antioxidant enzyme

The concentrations were calculated by comparing the optical density (OD) of the samples with a standard curve.

Oxidative Stress Measurement: The oxidative stress (OS) was assessed by calculating the oxidant/antioxidant balance ratio, with MDA used as the oxidant marker and SOD/GPx1 as antioxidant markers. OS was considered present if the balance was shifted towards the oxidative side at the time of evaluation, as compared to baseline, according to Pabón et al. [38]. A reduction in the oxidant/antioxidant balance ratio indicated the antioxidant efficacy of Ajwa Dates.

Statistical Analysis:

Data from the questionnaires were entered into the computer after thorough cleaning, and statistical analyses were performed using IBM SPSS Statistics, version 20. Descriptive statistics were employed to summarize quantitative variables. For normally distributed data, results were presented as mean ± standard deviation (SD), while non-normally distributed data were expressed as median and interquartile range (IQR; 1st quartile - 3rd quartile). Qualitative variables were summarized using numbers and percentages.

Comparisons were made between the intervention groups (Ajwa dates) and the control (non-dates) group, both before and after the intervention. The following statistical tests were applied:

- Pearson's Chi-square test (χ^2) for categorical variables and associations between groups.
- Z-test for qualitative data comparison.
- Paired t-test for comparing continuous data within groups between pre- and post-interventions.
- Independent t-test for comparing means of continuous data between two groups.
- Mann-Whitney U test for comparing medians between two groups (for non-normally distributed data).
- Wilcoxon Signed Ranks test for comparing non-uniformly distributed data within groups between pre- and post-interventions.

Additionally, relative risk (RR) was calculated to evaluate the risk differences between the groups. Statistical significance was set at a P-value < 0.05, and results with a P-value < 0.01 were considered highly significant.

Results

Effects of Date Regimens on Oxidative Stress Markers

Table 1 presents a highly significant improvement in both date regimens following the intervention period, as indicated by the balance ratios of Malondialdehyde to Superoxide Dismutase (MDA/SOD) and Malondialdehyde to Glutathione Peroxidase (MDA/GPx1), with p-values less than 0.001. In comparisons between the date regimens and the non-date group, significant improvements were observed in both MDA/SOD and MDA/GPx1 ratios, particularly for the MDA/SOD ratio, which showed high significance ($p < 0.01$). However, these ratios were not significantly different between the two date regimens.

Figure 2 illustrates the response rates based on improvements in oxidant to antioxidant ratio levels. More than three-quarters (82.0%) of children with autism in the date regimens exhibited significant improvements in their oxidant/antioxidant balance ratio as a result of the intervention. In contrast, less than half of the participants in the non-date regimen showed improvements in MDA/SOD and MDA/GPx1 ratios (40.5% and 47.6%, respectively).

Additionally, when analyzing the proportion of children who exhibited improved ratios versus those who did not benefit from the intervention, both date regimens demonstrated a significantly higher percentage of improvement ($p < 0.001$). In contrast, no improvements were observed in the non-date group (Table 2).

Evaluation of the Effect of Date Fruits on Oxidative Stress According to Autism Severity

Table 3 presents the percentage of improvement in the oxidant/antioxidant balance ratios, specifically the MDA/GPx1 and MDA/SOD ratios according to the reported DSM baseline severity. A highly significant improvement was observed in both moderate and severe cases of autism, as classified by the DSM-5 criteria. In contrast, no significant difference was found in the non-date group.

Table 1: Comparing median change of the oxidant/antioxidant Balance Ratio for children with autism according to the study regimen (Baseline levels vs. 1st evaluation post-intervention levels)

Parameter	children with autism			P value groups		
	Dates group (3 dates/day) n=47 Median (IQR)	Dates group (5 dates/day) n=42 Median (IQR)	Dates group (nondates) n=42 Median (IQR)	3 dates vs. non-dates	5 dates vs. non-dates	3 dates vs. 5 dates
Median Balance MDA/SOD						
Pre-intervention (baseline)	50.0 (29.7-86.9)	40.9 (24.7-74.0)	**38.5** (23.0-60.1)	0.102	0.570	0.29
Post-intervention (1st evaluation)	27.0 (15.0-45.0)	27.1 (13.2-51.3)	40.0 (**25.8**-93.9)	**0.02***	**0.04***	0.92
P value baseline and 1st evaluation	**<0.001****	**0.001****	0.050			
Median Balance MDA/GPX						
Pre-intervention (baseline)	164.0 (70.0-250.0)	164.1 (82.3-241.0)	100.0 60.0-179.0)	0.30	0.21	0.97
Post-intervention (1st evaluation)	64.2 (23.0-136.6)	63.8 (27.1-182.2)	100.0 (42.5-254.0)	**0.04***	0.04*	0.90
P value baseline and 1st evaluation	**<0.001****	**<0.001****	0.93			

Tests of significant were: Mann-Whitney test between two groups and Wilcoxon Signed Ranks test between pre and post of the same group, IQR: interquartile range (1st quartile - 3rd quartile), *significant < 0.05, **highly significant < 0.01

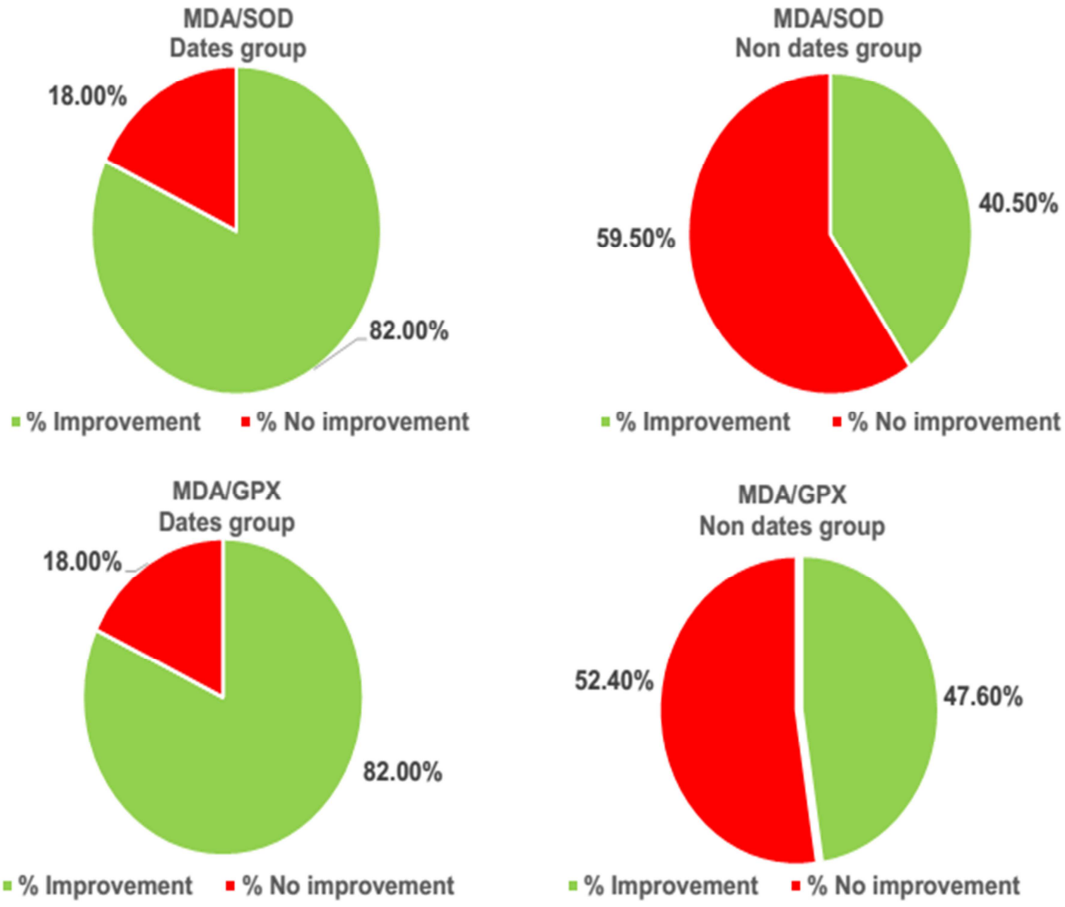

Figure (2): Percent distribution of the improved and non-improved oxidant/antioxidant Balance Ratio for autistic children

Table 2: Percent change of the oxidant/antioxidant Balance Ratio for children with autism according to the study regimen

Parameter	Children with autism			P value groups		
	Dates group (3 dates/day) n=47 No (%)	Dates group (5 dates/day) n=42 No (%)	Dates group (nondates) n=42 No (%)	3 dates vs. non- dates	5 dates vs. non- dates	3 dates vs. 5 dates
MDA/SOD						
• % Improved (n=90)	41 (87.2)	32 (76.2)	17 (40.5)	**<0.001****	**<0.001****	0.18
• % Non-improved (n=41)	6 (12.8)	10 (23.8)	25 (59.5)			
P value of z test	**<0.001****	**<0.001****	0.08			
P value between the three groups= <0.001**						
MDA/GPX						
• % Improved (n=93)	40 (85.1)	33 (78.6)	20 (47.6)	**<0.001****	**<0.003****	0.42
• % Non-improved (n=38)	7 (14.9)	9 (21.4)	22 (52.4)			
P value of z test	**<0.001****	**<0.001****	0.66			
P value between the three groups= <0.001**						

No improvement: sustained/deteriorated cases, tests of significance were: z test between proportions and X^2 test between the groups, ** highly significant < 0.01

Table 3: Percent change of the oxidant/antioxidant Balance Ratio for children with autism according to DSM5 levels of baseline severity

Parameter	Dates group (3 dates/day) n=47			Dates group (5 dates/day) n=42			Non dates n=42		
	Mild n=6 N (%)	Mod. n=21 N (%)	Severe n=20 N (%)	Mild n=5 N (%)	Mod. n=23 N (%)	Severe n=14 N (%)	Mild n=9 N (%)	Mod. n=18 N (%)	Severe n=15 N (%)
MDA/SOD									
• % Improved (n=90) (Post 3rd -Pre)	5 (83.3)	19 (90.5)	17 (85.0)	4 (80.0)	17 (73.9)	11 (78.6)	4 (44.4)	7 (38.9)	6 (40.0)
• % Non-improved (n=41)	1 (16.7)	2 (9.5)	3 (15.0)	1 (20.0)	6 (26.1)	3 (21.4)	5 (55.6)	11 (61.1)	9 (60.0)
P value of z test	**0.021***	**<0.001****	**<0.001****	0.057	**0.001****	**0.003****	0.638	0.184	0.271
P value of x^2 test	0.83			0.93			0.96		
MDA/ GPX									
• % Improved (n=93) (Post 3rd -Pre)	5 (83.3)	17 (81.0)	18 (90.0)	3 (60.0)	19 (78.6)	11 (82.6)	6 (66.7)	7 (38.9)	7 (46.7)
• % Non-improved (n=38)	1 (16.7)	4 (19.0)	2 (10.0)	2 (40.0)	4 (21.4)	3 (17.4)	3 (33.3)	11 (61.1)	8 (53.3)
P value of z test	**0.021***	**<0.001****	**<0.001****	0.529	**<0.001****	**0.003****	0.159	0.184	0.711
P value of x^2 test	0.71			0.10			0.39		

Tests of significant were: z test between two proportions and X^2 test between the groups, *significant < 0.05, **highly significant < 0.01

Assessing the Long-Term Effects of Date Fruits on Oxidative stress among children with Autism

In children with autism who completed their 3rd analysis following the date fruit regimen, there was a significant improvement in the median balance when comparing the pre-intervention (baseline) and the 3rd month samples for both date regimens. However, a deterioration in the median balance ratio was noted when comparing the 3rd month and the 6th month post-intervention samples. The oxidant/antioxidant median balance ratio three months after ceasing date intake was insignificantly lower than the baseline median level ($p > 0.05$), but it was highly significantly higher than the median observed after the 3rd month post-intervention ($p < 0.001$) (Figure 3).

Relation between Improved Balance of Oxidant / Antioxidant Ratio and Baseline Parameters

Table 4 illustrates the relationship between various baseline factors and the responses of children with autism following date intake, regardless of the type of date regimen. The observed improvements in both the oxidant/antioxidant ratios were not significantly different when comparing the two date groups. Additionally, the improvements in both ratios were not significantly associated with any of the studied baseline variables when compared to non-responders.

Relation between Baseline Factors and Responses of Children with Autism Across All Regimen Groups

Table 5 presents the relationship between various baseline factors and the responses of children with autism across all regimen groups. The observed improvements in the oxidant/antioxidant ratios were significantly associated with date intake. Specifically, the probability of improvement in the MDA/SOD level was over six times higher for children in the date regimen compared to those in the non-date regimen (RR = 6.7, 95% CI: 3.0–15.2). Similarly, the probability of improvement in the MDA/GPx1 level was five times higher for children in the date regimen than for those in the non-date regimen (RR = 5.0, 95% CI: 2.2–11.3). Notably, these improvements were not related to any of the other studied baseline factors.

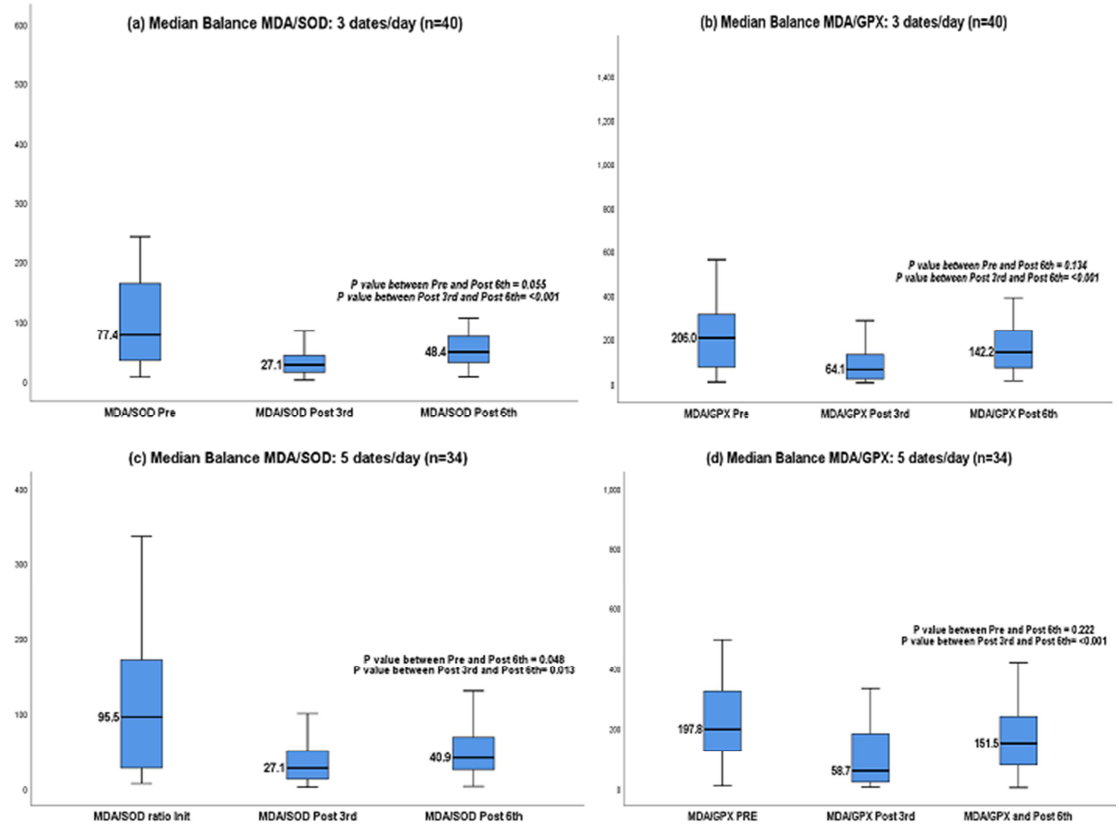

Figure (3): Median change of the oxidant/antioxidant Balance Ratio for children with autism who completed their third analysis according to dates' fruits regimen

Table 4: Characteristics of responders and non-responders in dates regimen groups

Parameter	MDA/SOD Level			MDA/GPX Level		
	Non respondents (not improved) (n=16) N (%)	Respondents (improved) (n=73) N (%)	RR (CI)	Non respondents (not improved) (n=16) N (%)	Respondents (improved) (n=73) N (%)	RR (CI)
Regimen type						
3 dates (n=47)	6 (37.5)	41 (56.2)	3 dates vs. 5 dates:	8 (50.0)	39 (53.4)	3 dates vs. 5 dates:
5 dates (n=42)	10 (62.5)	32 (43.8)	0.5 (0.2-1.4)	8 (50.0)	34 (46.6)	0.9 (0.3-2.6)
Age of the children						
< 6 years (n=44)	8 (50.0)	36 (49.3)	< 6 y. vs. ≥ 6 y.:	10 (62.5)	34 (46.6)	< 6 y. vs. ≥ 6 y.:
>= 6 years (n=45)	8 (50.0)	37 (50.7)	1.0 (0.4-3.0)	6 (37.5)	39 (53.4)	1.9 (0.6-5.8)
Baseline DSM5 severity						
Severe (n=34)	6 (37.5)	28 (38.4)	severe vs. not severe:	6 (37.5)	28 (38.4)	severe vs. not severe:
Not severe (n=55)	10 (62.5)	45 (61.6)	1.0 (0.3.3.0)	10 (62.5)	45 (61.6)	1.0 (0.3.3.0)
Baseline Risk of malnutrition						
At risk (n=40)	7 (43.8)	33 (45.2)	at risk vs. at potential risk: 0.9	8 (50.0)	32 (43.8)	at risk vs. at potential risk: 1.3
At potential risk (n=49)	9 (56.2)	40 (54.8)	(0.3-2.8)	8 (50.0)	41 (56.2)	(0.4-3.8)
Baseline Anthropometric						
Obese (n=50)	11 (68.8)	39 (53.4)	obese vs. non	9 (56.2)	41 (56.2)	obese vs. non
Non obese (n=39)	5 (31.2)	34 (46.6)	obese: 1.9 (0.6-6.1)	7 (43.8)	32 (43.8)	obese: 1.0 (0.3-3.0)

*Response based on improvement of oxidant to antioxidants ratio level, RR: relative risk, CI: confidence interval, tests of significant were: relative risk

Table 5: Characteristics of responders and non-responders in all regimen groups (improvement based on Changes of oxidant to antioxidants ratio level)

Parameter	MDA/SOD Level			MDA/GPX Level		
	Non respondents (not improved) (n=41) N (%)	Respondents (improved) (n=90) N (%)	RR (CI)	Non respondents (not improved) (n=38) N (%)	Respondents (improved) (n=93) N (%)	RR (CI)
Regimen type						
• Non dates (n=42)	25 (61.0)	17 (18.9)	non dates vs. dates:	20 (57.9)	20 (21.5)	non dates vs. dates:
• On dates (n=89)	16 (39.0)	73 (81.1)	6.7 (3.0-15.2)**	16 (42.1)	73 (78.5)	5.0 (2.2-11.3)**
Age of the children						
• < 6 years (n=64)	21 (51.2)	43 (47.8)	< 6 y. vs. ≥ 6 y.:	21 (55.3)	43 (46.2)	< 6 y. vs. ≥ 6 y.:
• >= 6 years (n=67)	20 (48.8)	47 (52.2)	1.2 (0.6-2.4)	17 (44.7)	50 (53.8)	1.4 (0.7-3.1)
Baseline DSM5 severity						
• Severe (n=49)	15 (36.6)	34 (37.8)	severe vs. not severe:	14 (36.8)	35 (37.6)	severe vs. not severe:
• Not severe (n=82)	26 (63.4)	56 (62.2)	1.0 (0.4-2.0)	24 (63.2)	58 (62.4)	1.0 (0.4-2.1)
Baseline Risk of malnutrition						
• At risk (n=61)	19 (46.3)	42 (46.7)	at risk vs. at potential risk:	20 (52.6)	41 (44.1)	at risk vs. at potential risk:
• At potential risk (n=70)	22 (53.7)	48 (53.3)	1.0 (0.5-2.1)	18 (47.4)	52 (55.9)	1.4 (0.7-3.0)
Baseline Anthropometric						
• Obese (n=36)	15 (36.6)	21 (23.3)	obese vs. non	11 (28.9)	25 (26.9)	obese vs. non
• Non obese (n=95)	26 (63.4)	69 (76.7)	obese: 1.9 (0.9-4.2)	27 (71.1)	68 (73.1)	obese: 1.1 (0.5-2.6)

Discussion

The documented brain's vulnerability to oxidative stress in subjects with ASD, and the subsequent damage of neuronal cells necessitates the development of effective therapeutic strategies. Existing pharmacological treatments often prove inadequate and are associated with adverse effects. Plant-derived bioactive compounds may offer a promising alternative, owing to their antioxidant and neuroprotective characteristics, which can be utilized in the treatment and management of various neuropsychiatric, neurodevelopmental, and neurodegenerative disorders [39 & 40]

Date fruit as a potential antioxidant

The date fruit (Phoenix dactylifera), particularly rich in polyphenols, flavonoids, micronutrients, and dietary fiber [27], has the potential to mitigate oxidative stress.

This study is a randomized controlled trial aimed at evaluating the efficacy of dates in reducing oxidative stress through evaluation of blood levels of three oxidative stress markers—MDA, GPx1, and SOD—in children with Autism Spectrum Disorder (ASD). Our findings show significant improvements in oxidative stress balance in the groups consuming dates compared to the non-date group. Both date groups demonstrated reductions in oxidative stress, with improvements observed in MDA, SOD, and GPx1 levels as a result of the intervention. These results align with [41], who found that date palm extract administration significantly decreased MDA levels and increased SOD levels in rats subjected to oxidative stress.

Mechanisms Behind Date's Antioxidant Effects

The antioxidant effects of dates may be attributed to their polyphenol content [27], which neutralizes free radicals through their hydroxyl groups attached to benzene rings. These compounds effectively scavenge superoxide anions, singlet oxygen, and lipid peroxyl radicals. Furthermore, the vitamin C content in dates helps combat oxidative stress via electron donation, diminishing reactive oxygen, nitrogen, and sulfur species, and regenerating other antioxidants such as alpha-tocopherol [42 & 43]. Vitamin C also prevents lipid peroxidation caused by peroxide radicals. It can diminish reactive species of oxygen, nitrogen, and sulfur radicals, and restore other antioxidants in the body, including alpha-tocopherol. Furthermore, Vitamin C plays a significant role in preventing lipid peroxidation caused by peroxide radicals [44,45].

The antioxidant properties of dates make them useful in managing and potentially preventing a range of diseases driven by oxidative stress and inflammation. However, the benefits need to be balanced with considerations like the high sugar and potassium content in certain populations (e.g., diabetic or renal patients). Dates can be particularly beneficial in diseases like cardiovascular conditions, diabetes, neurodegenerative disorders, and chronic inflammatory diseases. While dates have antioxidant and anti-inflammatory properties that could benefit hemodialysis patients, their high potassium content poses a significant risk. Therefore, their inclusion in practice guidelines should be highly individualized, [46 &47] with limited portion sizes and close monitoring of potassium levels. Raising awareness about the health benefits of dates requires a multi-faceted approach [48 & 49] that is proven to be effective in Egypt to involve the healthcare community [50 & 51], public health campaigns [52], communication for behavioral development [53 &54], educational programs [55], social marketing [56], and grassroots outreach [57]. By using various platforms, from digital campaigns to in-person workshops, you can help educate both the public and healthcare professionals on how dates can be a powerful tool in managing diseases linked to oxidative stress.

Effect of Date Intake on Autism Severity Effect of Date Intake on Autism Severity

In the present study, improved oxidative balance correlated ASD severity as measured by (CARS and

DSM5 scores. In both dates groups, the improvements were particularly pronounced in children with moderate to severe autism. This suggests that children with more pronounced ASD symptoms might benefit more from antioxidant-rich interventions like date consumption. The oxidative stress mechanisms may be more active or dysregulated in children with severe autism, making antioxidant interventions particularly effective. This aligns with Jardim and his colleagues [58] who emphasized the role of dietary polyphenols in alleviating ASD symptoms.

Role of Polyphenols in Autism

Previous studies support the potential of polyphenols in reducing autism symptoms. In a pilot study involving 50 children with ASD, [59] reported improvements in communication, concentration, and a decrease in atypical behaviors following polyphenol intake. Similarly, Tsilioni et al, & Amadi et al., [60 &61] found that polyphenol supplementation significantly reduced serum pro-inflammatory cytokines, IL-6, and TNF, suggesting a reduction in neuroinflammation, a key factor in ASD pathogenesis.

Long-Term Efficacy of Date Intake on improvements of oxidative balance

The findings of the present study indicate that dates serve as an effective antioxidant source, exhibiting time-dependent efficacy in reducing oxidative stress. A significant improvement in median balance was observed when comparing the balance ratios before and after three months of date consumption, regardless of the dietary regimen. However, the post-sixth month balance ratio remained better than the pre-intervention ratio, but the difference was insignificant. This is aligning with [62] "oxidative stress compensation model," which suggests that dietary antioxidants have limited long-term effects on oxidative stress as the body compensates by producing more superoxide [63].

Non-Responders and Influence of Baseline Factors

The presence of non-responders—individuals who do not exhibit significant improvements despite undergoing the same intervention as others—is a crucial factor. For healthcare providers, recognizing that not all children with autism will respond equally to dates - antioxidant interventions- is critical. Non-responders should not be viewed as failures of the intervention but rather as individuals who may require a different approach, whether through adjusting the dosage, duration, or combination of dietary antioxidants.

Understanding the reasons behind this variability is essential for optimizing dietary interventions and personalizing nutrition strategies for ASD. In the present study, some children with autism did not show significant improvements in their oxidative stress markers (MDA/SOD and MDA/GPx1 ratios) following the date fruit intervention. This phenomenon of non-response can be attributed to several factors. Differences in age, sex, disease severity, and baseline levels of oxidative stress markers can all influence how an individual responds to dates intake. However, statistical analysis of the current findings did not prove this theory. It has been recognized that none of these factors influence the response to the intervention regimen. This variation in response could be explained by the initial disparity of methylation density of certain gene promoters among children before receiving the regimen [27]

Children with varying baseline oxidative stress levels may require different doses or durations of the intervention to detect measurable benefits. Moreover, the variability observed among individuals in their responses to the studied regimens can be attributed to the differences in their absorption and metabolism. This suggestion was supported by previous research studies on carotenoids [64]. Manach et al. highlighted the role of gene variants in carotenoid absorption and metabolism, suggesting that personalized nutrition may be needed to account for individual differences. Similarly, Jungert and Frank (2021) [65] observed high intra- and inter-individual variation in antioxidant biomarkers, emphasizing the influence of intrinsic and extrinsic factors.

The influence of baseline factors such as body mass index (BMI) significantly affects the outcomes of dietary interventions [66]. In this study, baseline factors did not show a clear association with the improvements in oxidative stress markers across the group of children who responded well to date intake. Another explanation is that children with higher baseline oxidative stress may experience more noticeable improvements after intervention, as their bodies are in greater need of redox balance restoration. Those with milder oxidative stress may already be closer to their oxidative stress set point and, therefore, show less dramatic changes. Younger children might metabolize antioxidants differently from older ones due to developmental differences in metabolic and enzymatic pathways. It is well known that several factors influence child development in all aspects [67-71]. Personalized strategies, perhaps involving genetic testing or gut microbiome analysis, could eventually become standard practice to maximize the efficacy of dietary antioxidants like those found in dates. Pairing date consumption with other antioxidant-rich foods or supplements could also address non-response. For example, combining polyphenol-rich dates with Vitamin C or tocopherols may enhance the overall antioxidant defense system.

Strengths

The study presents statistically significant findings, demonstrating that Ajwa date consumption leads to notable improvements in oxidative stress markers among children with Autism Spectrum Disorder (ASD). The use of a randomized controlled trial design enhances the reliability of the results by minimizing bias, ensuring that observed effects can be attributed to the date intervention. Additionally, the research assesses the effectiveness of date consumption across different severity levels of autism, allowing for a more nuanced understanding of its effects on various populations within ASD. The investigation of both short-term and long-term effects provides valuable insights into the sustained benefits or drawbacks of dietary changes in managing oxidative stress. Furthermore, the use of appropriate statistical analyses ensures that conclusions drawn are based on solid evidence, enhancing the study's credibility.

Limitations

Despite its strengths, the study has some limitations. While the sample size is substantial, a larger cohort could improve the generalizability of the findings and provide more robust data regarding the effects of date

consumption. The duration of the intervention and follow-up may not be sufficient to fully understand the long-term implications of date fruit intake on oxidative stress markers and ASD symptoms. Additionally, focusing solely on Ajwa dates limits the ability to generalize findings to other date varieties or dietary interventions, potentially narrowing the scope of dietary recommendations for children with ASD. Although baseline characteristics were analyzed, the lack of significant associations with improvements in oxidative stress markers suggests that other unmeasured factors could influence outcomes. Furthermore, if dietary adherence was self-reported by parents or caregivers, this could introduce bias or inaccuracies in assessing compliance with the date regimen. Lastly, relying on specific biomarkers (MDA, SOD, GPX1) may not capture the full complexity of oxidative stress and its implications for children with ASD, indicating a need for a broader range of biomarkers in future research.

Conclusions and recommendations

Based on the results, the consumption of Ajwa dates significantly improved oxidative stress markers in children with Autism Spectrum Disorder (ASD), as evidenced by enhanced oxidant/antioxidant balance ratios (MDA/SOD and MDA/GPX1). Both moderate and severe cases of autism showed substantial improvements, highlighting the potential of date consumption as a beneficial dietary intervention. Furthermore, while short-term benefits were evident, the long-term effects demonstrated a slight decline in the oxidant/antioxidant balance after cessation of date intake, suggesting that continued consumption may be necessary to maintain these benefits. Additionally, the improvements in oxidative stress markers were significantly associated with date intake, regardless of baseline characteristics, underscoring the potential role of dietary modifications in managing oxidative stress in children with ASD. Overall, the findings support the inclusion of Ajwa dates in dietary recommendations for children with ASD to mitigate oxidative stress and improve overall health outcomes. This is necessitated to raise mothers' awareness of the advantages of palm dates from early childhood or even during pregnancy, utilizing community-based strategies that have been demonstrated to effectively promote behavioral changes Metwally and her colleagues [48 & 55]. Non-responders in clinical trials on the antioxidant effects of dates highlight the importance of accounting for individual variability in nutrient absorption, metabolism, and genetic background. The influence of baseline factors such as age, initial oxidative stress levels, and developmental stage further underscores the need for personalized approaches in dietary interventions. By tailoring antioxidant-rich diets to individual needs, future research and clinical practice can better harness the potential of natural antioxidants like dates for disease prevention and management.

Ethics approval and consent to participate

The study was conducted in accordance with the ethical standards set forth by the Medical Research Ethics Committee of the National Research Center (Approval Ethical Number: 19203). Additionally, the study was registered with the US National Institutes of Health at ClinicalTrials.gov (#NCT04261595), with Protocol ID: 12060158, and the initial registration date was 10/02/2020. Written informed consent was obtained from the parents or caregivers of all participants after thoroughly explaining the study's objectives and potential benefits for their children. The informed consent process ensured that participants fully understood the study and their rights before agreeing to participate. This study adhered to the International Ethical Guidelines for Biomedical Research Involving Human Subjects [72] and followed specific recommendations to ensure that patients and their guardians understood the purpose and procedures of the research. Special attention was given to aligning with the recommendations for obtaining informed consent based on the perceptions of Egyptian patients and guardians [73]. This approach emphasized clear communication and the safeguarding of participants' rights throughout the research process.

Competing interests

All authors report no conflict of interest. “No financial or non-financial benefits have been received or will be received from any party related directly or indirectly to the subject of this manuscript.

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Availability of data and materials

All data generated or analyzed during this study are available upon request from the corresponding author.

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