*Original Research*

# An Open-Label Pilot Study of a Formulation Containing the Anti-Inflammatory Flavonoid Luteolin and Its Effects on Behavior in Children With Autism Spectrum Disorders

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## ABSTRACT

**Background:** Accumulating evidence suggests an association between autism spectrum disorders (ASD) and inflammation in brain regions related to cognitive function. The natural flavonoid luteolin has antioxidant, anti-inflammatory, mast cell–blocking, and neuroprotective effects. It was shown to improve cognitive performance in a mouse model of ASD, but its effect in humans has not been adequately studied.

**Objectives:** The goal of this study was to assess the effectiveness and tolerability in white children with ASD of a dietary supplement containing 2 flavonoids (>95% pure), luteolin (100 mg/capsule, from chamomile) and quercetin (70 mg/capsule), and the quercetin glycoside rutin (30 mg/capsule) from the Sophora japonica leaf, formulated in olive kernel oil to increase oral absorption.

**Methods:** Fifty children (4–10 years old; 42 boys and 8 girls) with ASD were enrolled in a 26-week, prospective, open-label trial at the 2nd University Department of Psychiatry at "Attikon" General Hospital, Athens, Greece. Children were referred for the study by their respective physicians or came from the practice of the senior author. ASD diagnosis by clinical assessment was based on the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision,* symptom list and corroborated by using the Autism Diagnostic Observation Schedule. The dose of the study formulation used was 1 capsule per 10 kg weight per day with food. The primary outcome measures were the age-equivalent scores in the Vineland Adaptive Behavior Scales domains. Secondary outcomes included the Aberrant Behavior Checklist, the Autism Treatment Evaluation Checklist, and the Clinical Global Impression–Improvement score. Data were measured at baseline, week 18, and week 26. Parents were interviewed for any possible improvements they noticed and instructed to report any unusual adverse events.

**Results:** A total of 40 children completed the protocol. There was a significant improvement in adaptive functioning as measured by using the VABS age-equivalent scores (8.43 months in the communication domain, 7.17 months in daily living skills, and 8 months in the social domain; $P < 0.005$), as well as in overall behavior as indicated by the reduction (26.6%–34.8%) in Aberrant Behavior Checklist subscale scores. Age, sex, and history of allergies had no effect on the results, whereas the initial level of functioning or difficulty did predict the final outcome in most of the measures used. There was a transient (1–8 weeks) increased irritability in 27 of the 50 participants.

**Conclusions:** These results are encouraging in that the combination of the flavonoids luteolin and quercetin seemed to be effective in reducing ASD symptoms, with no major adverse effects. ClinicalTrials.gov identifier: NCT01847521. (*Clin Ther*. 2013;35:592–602) 

**Key words:** ASD, luteolin, flavonoids, inflammation, brain.

## INTRODUCTION

Autism spectrum disorder (ASD) is a life-long condition characterized by marked impairment in social communication and language, as well as repetitive/restricted behaviors.[1,2] A steady increase in its preva-

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lence has been well documented in the past 20 years, with recent findings ranging from 1.13% in the last Centers for Disease Control and Prevention study[3] (in 2008) to 2.64%.[4] The lack of any definitive pathogenesis has prevented the development of effective treatments for the core symptoms of ASD.[5,6] The current use of medications addresses only specific secondary behavioral symptoms of the disorder. Moreover, recent studies have shown that use of selective serotonin reuptake inhibitor antidepressants[7] and some antipsychotic agents[8] may actually worsen ASD symptoms. Although risperidone and aripiprazole are 2 antipsychotic agents approved for use in children with ASD, these medications only address the disruptive, aggressive, and self-mutilative behaviors and not the core symptoms of the disorder. Only recently has research tried to translate behavioral findings to possible targets for pharmacologic agents in an attempt to address the core symptoms of ASD.[9–11]

Increasing evidence indicates that brain inflammation is important in the pathogenesis of neuropsychiatric disorders,[12,13] including at least a significant proportion of subjects with ASD.[14–16] "Allergic" issues, especially food intolerance and eczema, are often present among children with ASD.[17–20] Mast cells, which are implicated in both allergic and inflammatory reactions, are activated in autism,[21] and the prevalence of ASD seems to be 10 times higher among children with mastocytosis.[22]

Natural flavonoids, such as luteolin and quercetin, exhibit potent antioxidant and anti-inflammatory activities,[23] inhibit the release of inflammatory mediators from human mast cells,[24] and reduce maternal interleukin 6–induced autism-like behavioral deficits related to social interactions in mice.[25] However, these flavonoids have not been adequately studied in children. A case series of children with ASD in the United States (37 children, 4–14 years old) who took a dietary supplement containing luteolin and quercetin* for 4 months reported gains in eye contact and improvements in attention in 50% of subjects and social interaction in 25% of subjects.[26] However, no data were reported regarding subject characteristics, no baseline measurements were taken, and the reported gains were solely based on parental impression, with no use of objective instruments. We conducted here an open-label study by using validated instruments to assess the tolerability and effectiveness of the same trial formulation in Greek white children in 2 age groups (4–6 years old and 7–10 years old) to try and establish any correlation with age, severity of symptoms or history of allergic problems.

*Trademark: NeuroProtek® (Algonot, LLC, Sarasota, Florida).

## SUBJECTS AND METHODS

Children were referred to the Athens University "Attikon" 2nd Psychiatric Clinic for ASD from various professionals, as well as from the private practice of the senior author, from around Athens, Greece. The study was announced at ASD support groups and at the ASD clinic. No other centers were involved. The parents of all subjects were informed of the study's aims, including risks versus benefits of participating and not participating as well as the inclusion and exclusion criteria. They provided written consent for participation in the study after being informed of all details of the study. The study was approved by the Ethics Committee of "Attikon" General Hospital, Athens, Greece.

Fifty white children (42 boys and 8 girls; 4–10 years of age) with ASD were enrolled consecutively in this 26-week, prospective, open-label trial after meeting the inclusion and exclusion criteria. They were divided into 2 age groups (4–6 years old, n = 25; 7–10 years old, n = 25). Participants had already been diagnosed with ASD based on clinical assessments, and this diagnosis was corroborated at the 'Attikon' clinic by meeting the cutoff scores on both the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision,* symptom list[27] and the Autism Diagnostic Observation Schedule algorithm.[28] All children were medication naive and had never received the study luteolin formulation. None of the referred subjects met any of the exclusion criteria set (ie, any medical condition likely to be etiological for ASD [eg, Fragile X syndrome, tuberous sclerosis], any neurologic disorder involving pathology above the brain stem [other than uncomplicated nonfocal epilepsy], any evidence of probable neonatal brain damage, mastocytosis [including urticaria pigmentosa] and a history of systemic inflammatory diseases).

Children were administered the dietary formulation made by a Good Manufacturing Practices–certified facility (Tishcon Laboratories, Long Island, New York) under contract from Algonot, LLC (Sarasota, Florida; www.algonot.com). The study formulation contains 2 flavonoids (>95% pure), luteolin (100 mg/capsule, from chamomile) and quercetin (70 mg/capsule), and the quercetin glycoside rutin (30 mg/capsule) from the

Sophora japonica leaf; it is free of all known allergens. To increase the poor oral absorption of the lipid-soluble flavonoids,[29] they were formulated in microspheres by mixing them in olive kernel oil of low oleic acid and water content (< 0.01%). The dose used was 1 softgel capsule per 10 kg (22 lb) weight per day with food for 26 weeks.

Apart from the diagnostic evaluation, the assessment also included a thorough medical evaluation comprising a physical examination and health history (including a review of allergic and gastrointestinal symptoms, as well as any food allergies or food intolerance). There were no subjects with ragweed sensitivities, and there has only been 1 reported instance of an allergic response to chamomile in a multisensitive individual.[30]

The current study was not performed to satisfy any regulatory request or requirement. All concurrent interventions were thoroughly noted (type and hours), and the same was done at all visits. After meeting screening criteria, subjects were evaluated at the baseline visit, mid-trial visit at 18 weeks, and final visit at 26 weeks.

The primary outcomes were the age-equivalent scores in the 3 domains of the Vineland Adaptive Behavior Scales (VABS).[31] The VABS was chosen because the impact of an agent on adaptive functioning in real life is even more important for obtaining a better quality of life than just alleviation of some symptoms.[32] Secondary outcomes included the Aberrant Behavior Checklist (ABC),[33] the Autism Treatment Evaluation Checklist (ATEC),[34] and the Clinical Global Impression–Improvement score (CGI-I).[35]

The VABS is a well-validated parent interview that focuses on the child's actual functional skills. It consists of 3 domains: communication, daily living skills, and socialization. For children up to the age of 6 years, there is a fourth domain (motor skills). The raw scores from the interview can be also expressed through a standardization process as an age-equivalent score and a standard score compared with those of the subject's peers. The standard scores of the first 3 domains generate an adaptive behavior composite score, as a global estimate of functioning. Carter et al[36] also presented supplementary special norms for individuals with autism. Various studies have chosen different scores from the VABS as an outcome measure.[37] Although standard scores could be more useful in subject characterization, their use as an outcome measure has been proven to be less sensitive due to floor effects and reduced variability, especially in short time periods, and thus these scores underestimate change.[37] Conversely, scores of special norms tend to overestimate change, as a small increase in a raw score can produce a big improvement in special norm percentile rank.[36] Thus, raw scores and age-equivalent scores seem to be the most appropriate for use as outcome measures,[36,37] with the latter being more easily interpreted as change over time.[37] Furthermore, age-equivalent scores can be used to generate an Adaptive Behavior Index (ABI) as a measurement of the child's adaptive growth by using the formula shown below[37]:

$$\text{ABI} = \frac{\text{AE}}{\text{CA}} \times 100 \qquad \begin{array}{l}\text{AE} = \text{age-equivalent} \\ \text{CA} = \text{chronological age}\end{array}$$

In assessing the effectiveness of an intervention with the use of ABIs, we can compare the difference between the observed adaptive behavior growth (ABI at end point) and the projected growth established at baseline (ABI at baseline) to see if the observed gains were greater than those expected just by maturation.

To explore other possible effects of the formulation not captured from the aforementioned instruments, we chose to record any other benefits observed and reported by the parents during its use. For this, the primary clinician (K.F.) conducted telephone or in-person interviews of the parents, independently of the assessing clinician (A.T.), to discuss the possible gains of the child. CGI-I was also independently coded by the primary clinician with personal assessments as well as with information gathered by parents and, in the majority of cases, by the subjects' trainers.

Compliance was monitored by softgel capsule count and the parents' assurance that the capsules had actually been taken at each visit; in case of a capsule count <85% of the prescribed dosage at midterm and at the end of the study, the subject was excluded from the final analysis. Adverse events were systematically recorded on an adverse event form by using scales indicating severity, relationship to the study procedures, action taken, and any therapy required.

### Statistical Analysis

The data were extracted from the relevant case-report forms and entered into a statistical package. Before analysis, the data were validated and inspected for outliers and spurious data. The effect of each outcome (VABS domains, ABC subscales, and ATEC subscales)

in time was investigated by using a general linear model for repeated measurements. The effects of sex, age, allergy, and the level of the corresponding baseline score (as defined if the score for this outcome was above or below the median), and their interaction with time were also then investigated by incorporating each variable, independently, as a between-subject factor in the model. Adjustment of the significance level according to the number of tests across domains/subscales of each outcome (multiplicity effect) was not considered because we were interested in exploring each domain/subscale independently, and no global hypothesis (ie, all domains show no improvement) was tested.[38] The relationship between the relative change (from baseline to week 26) in VABS raw scores (ΔVABS), consideration of VABS domain as a response variable, and the relative change in the 5 ABC subscales (which were considered as explanatory variables) was investigated by using a multivariate regression model.

A result was considered significant at a $P$ value $<0.05$. The analysis was performed by using SPSS version 19 (IBM SPSS Statistics, IBM Corporation, Armonk, New York).

## RESULTS

A total of 40 children (35 boys and 5 girls) completed the protocol by December 2012. Six children withdrew from the study due to the increased irritability caused by the formulation (2 of them were good responders to the formulation; 2 others had not responded until the mid-visit); 1 showed poor compliance; parents were unable to administer the capsule or its content in 2 children; and in 1 case, the parents decided not to participate after completing the baseline assessment. The demographic and baseline clinical characteristics of the compliant children are provided in **Table I**. They were predominately males (88%) and were evenly distributed in the 2 age groups (4–6 years and 6–10 years), with a mean age of 80 months and no statistical difference between the age of male and female children. It is noteworthy that 55% of children had reported allergies or possible allergic symptoms. Participants were receiving various special behavioral interventions with a mean intensity of 7.75 hours per week (2–20 hours per week). No major changes in the hours of treatment were noted throughout the study.

The responses in primary outcomes and the comparisons in time are shown in **Table II**. The statistical analysis indicated that the changes in raw and age-equivalent scores were significant ($P < 0.05$) for all domains, except the communication raw score ($P = 0.08$). However, the changes in the VABS standard scores were significant ($P < 0.01$) only for the social domain. The VABS composite score was also significantly higher at the end of the study. Effect sizes were in the medium range for the age-equivalent scores and the statistically significant raw scores (0.35–0.44) but lower for the standard score of the social domain (0.24). These data indicate a significant positive effect on the adaptive functioning of the subjects over an average 7-month period; they gained 8.43 months in the communication domain, 7.17 months in daily living skills, and 8 months in the social domain. This conclusion is further strengthened by the fact that ABI scores for all domains were significantly higher at the end point (effect sizes, 0.20–0.22), indicating that the observed gains were

**Table I. Demographic and baseline clinical characteristics of the compliant study subjects (N = 40).**

| Characteristic | Value |
|---|---|
| Sex, no. | |
| Male | 35 |
| Female | 5 |
| Age, no. | |
| 4–6 y | 19 |
| 6–10 y | 21 |
| Mean (SD) age, mon | 79.94 (20.08) |
| Allergy, no. | |
| Yes | 11 |
| Maybe | 11 |
| No | 18 |
| Gastrointestinal symptoms, no. | |
| Yes | 11 |
| Maybe | 20 |
| No | 9 |
| Route of administration, no. | |
| Capsule | 23 |
| Contents of capsule only | 17 |
| Hours/week of special intervention | |
| Range | 2–20 |
| Mean | 7.75 |

Table II. Effectiveness of the study luteolin formulation in children with autism spectrum disorders: primary outcomes.

| Vineland Adaptive Behavior Scales | 0 Week | 26 Weeks | Change | Effect Size* | *P* |
|---|---|---|---|---|---|
| Communication | | | | | |
| Raw score | 52.31 (27.18) | 57.00 (31.47) | 4.69 (8.54) | 0.17 | 0.08 |
| Standard score | 56.54 (21.70) | 58.95 (26.80) | 2.41 (11.31) | 0.11 | 0.10 |
| Age-equivalent | 34.56 (24.28) | 43.00 (31.37) | 8.43 (9.61) | 0.35 | <0.01 |
| Adaptive Behavior Index† | 44.23 (28.42) | 50.33 (35.81) | 6.10 (10.57) | 0.21 | <0.01 |
| Daily living skills | | | | | |
| Raw score | 61.97 (23.84) | 72.08 (26.97) | 10.12 (8.4) | 0.42 | <0.01 |
| Standard score | 56.17 (17.75) | 58.80 (22.46) | 2.63 (8.74) | 0.15 | 0.12 |
| Age-equivalent | 39.35 (16.26) | 46.53 (20.70) | 7.17 (6.78) | 0.44 | <0.01 |
| Adaptive Behavior Index† | 50.16 (20.00) | 54.16 (22.90) | 4.0 (7.43) | 0.20 | <0.01 |
| Social | | | | | |
| Raw score | 52.31 (18.12) | 59.77 (21.04) | 7.46 (5.60 | 0.41 | <0.01 |
| Standard score | 63.87 (14.64) | 67.40 (19.03) | 3.53 (6.53) | 0.24 | 0.01 |
| Age-equivalent | 34.58 (18.93) | 42.58 (25.44) | 8.0 (10.18) | 0.42 | <0.01 |
| Adaptive Behavior Index† | 44.73 (23.62) | 50.26 (30.67) | 5.53 (11.43) | 0.23 | <0.01 |
| Composite score | 52.21 (17.04) | 56.00 (22.30) | 3.79 (7.23) | 0.22 | <0.01 |

*Change (26 week – 0 week)/baseline SD.
†(Age-equivalent/chronological age) × 100

greater (4.0%–6.10%) than those expected by the maturation process alone.

Table III displays the secondary behavioral outcomes and their comparisons in time. All ABC subscales (hyperactivity and noncompliance, irritability and agitation, lethargy and social withdrawal, stereotypic behavior) showed significance ($P < 0.01$) in time, except 1 subscale (inappropriate speech), which showed marginal significance ($P = 0.04$). The time effect was predominant between baseline and week 26. Effect sizes ranged from large for hyperactivity (−0.86) to medium for the rest of the subscales (−0.42 to −0.51). Conversely, ATEC derived significant results only for the subscale health/physical/behavior ($P = 0.01$), which was mainly due to the change in response at week 26. The CGI-I score indicated, on average, a minor improvement for both time periods (weeks 18 and 26), and the CGI-I score was not significantly different between the 2 periods (2.94 [0.86] vs 2.77 [0.83]; $P = 0.14$). However, 15 (37.5%) of 40 children were much improved or very much improved according to the primary clinician. The amount of received hours of special treatment was not correlated with the improvement shown by the CGI-I, indicating that the difference in the intensity of receiving treatment could not account for the differences in the gains observed.

Changes in age-equivalent scores from all 3 VABS domains were significantly affected ($P < 0.01$) by the baseline corresponding values, with subjects who had initial adaptive functioning above the median showing more gains than those scoring below the median initially (Figure). Sex, age, and the presence of allergies had no significant effect on the changes in adaptive functioning. The same pattern emerged if we performed the analysis by using the ABI from all domains, the raw scores from the social domain, and the standard scores from the communication or the daily living skills domain. These results indicate that only initial adaptive function level influenced the final result in that more-able children responded better to the study formulation, whereas age, sex, and the presence of allergies were not good predictors for the final outcome.

From the scales assessing behavioral difficulties, only ATEC speech/language/communication, ABC stereo-

Table III. Effectiveness of the study luteolin formulation in children with with autism spectrum disorders: secondary outcomes.

| Tests | 0 Week | 18 Weeks | 26 Weeks | *P* (0 Versus 18 Weeks) | *P* (18 Versus 26 Weeks) | *P* (0 Versus 26 Weeks) | Effect Size* (0 Versus 26 Weeks) | *P* Overall |
|---|---|---|---|---|---|---|---|---|
| ABC | | | | | | | | |
| Hyperactivity | 20.47 (8.25) | 17.11 (8.96) | 13.33 (7.43) | 0.03 | <0.01 | <0.01 | –0.86 | <0.01 |
| Irritability | 13.16 (7.49) | 11.38 (7.17) | 9.41 (7.06) | 0.10 | <0.01 | <0.01 | −0.50 | <0.01 |
| Lethargy | 13.94 (7.83) | 11.44 (7.94) | 9.91 (7.30) | 0.02 | 0.10 | <0.01 | −0.51 | <0.01 |
| Stereotypic behavior | 5.94 (3.71) | 6.00 (4.56) | 4.36 (3.85) | 0.99 | 0.01 | 0.01 | −0.42 | <0.01 |
| Inappropriate speech | 3.08 (2.30) | 2.75 (2.29) | 2.25 (1.94) | 0.96 | 0.06 | 0.07 | −0.36 | 0.04 |
| ATEC | | | | | | | | |
| Speech/language/ communication | 12.75 (7.89) | 11.75 (7.41) | 11.30 (7.31) | 0.38 | 0.99 | 0.50 | −0.18 | 0.24 |
| Sociability | 13.80 (7.46) | 13.47 (7.32) | 12.77 (7.98) | 0.99 | 0.99 | 0.99 | −0.13 | 0.53 |
| Sensory/cognitive awareness | 13.50 (8.30) | 12.13 (7.97) | 12.25 (9.38) | 0.08 | 0.99 | 0.99 | −0.15 | 0.36 |
| Health/physical/ behavior | 18.38 (8.19) | 17.19 (8.53) | 14.94 (8.82) | 0.79 | 0.03 | 0.01 | −0.42 | 0.01 |
| Clinical Global Impression–Improvement | NA† | 2.94 (0.86) | 2.77 (0.83) | NA† | 0.14 | NA† | | 0.14 |

ABC = Aberrant Behavior Checklist; ATEC = Autism Treatment Evaluation Checklist.
*Change (26 weeks – 0 week)/baseline SD.
†CGI-Improvement was not administered at baseline.

type, and ABC hyperactivity were found to follow the same pattern, and children with more problems (ie, higher scores) had more gains (ie, larger score reduction). This finding could mean that children with more stereotypies and hyperactivity, as well as more language and communication problems, are also better responders.

To determine if the adaptive functioning gains (differences in VABS raw scores [ΔVABS]) were secondary to the behavioral improvement observed (differences in the ABC subscales scores), we examined whether each ΔVABS domain is related to the ΔA$\beta$C subscales. None of the ΔVABS domains were related to ΔA$\beta$C subscales ($P \geq 0.05$). The only exception was the effect of reduction of lethargy subscale on the observed difference in the VABS social raw score (P = 0.016) (observed Pearson correlation between the 2 variables, $r = -0.28$). Thus, we cannot assume that the improvement in adaptation was mainly the result of the behavioral improvements obtained with this formulation.

In one half of the subjects, their parents reported various improvements in speech (articulation, vocabulary, length of sentences, and words used meaningfully). As also indicated by the VABS social domain scores, parents reported better sociability in almost one half of the subjects (19 of 40). Children were reported to be more "cooperative" (17 of 40) and with better and prolonged concentration abilities (12 of 40). Gains were also reported for receptive language abilities (30%) and in general communication intention and abilities (20%). Less prevalent gains (10%–15%) were noted in eye contact, gastrointestinal symptoms, sleep, and stereotypies.

The most frequent adverse effect was an increased irritability (27 of 50), of various durations, that led to study withdrawal in 6 participants. Irritability was usually transient, lasting 1 to 8 weeks in 66% of cases; in most cases, it started 2 to 7 days after the formulation administration (20 of 27). In 1 subject, however, it became apparent 2 months after the treatment onset. In some cases, it was addressed with reduction or splitting the dosage over the day. Less frequent adverse events are recorded in **Table IV**.

## DISCUSSION

The current results indicate a statistically significant benefit of the luteolin study formulation both in adaptive functioning (as shown by the the VABS scores) and in the overall behavior (as indicated by the ABC). This

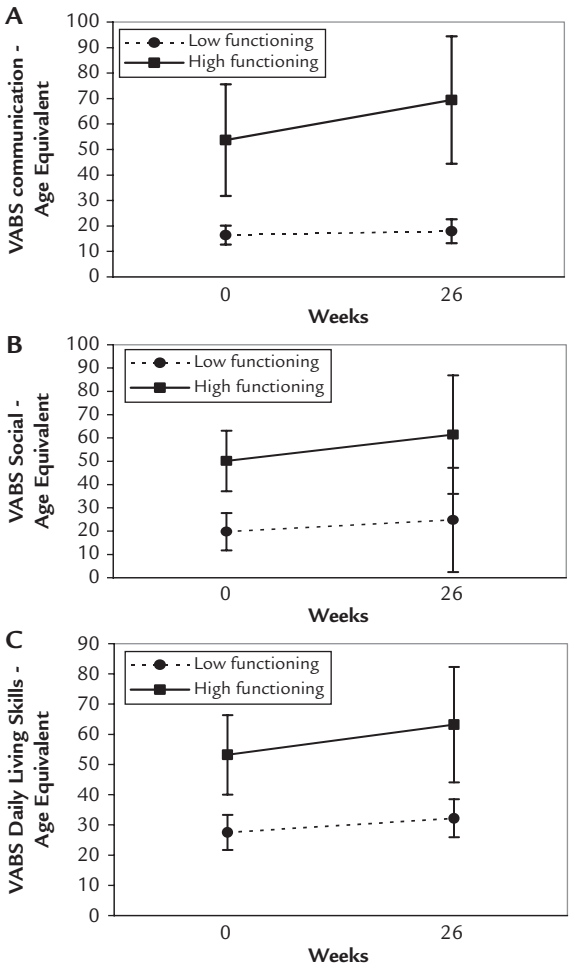


Figure. Effect of baseline functioning on age-equivalent scores from the 3 domains of the Vineland Adaptive Behavior Scales (VABS): (A) communication, (B) social, and (C) daily living skills. Low functioning = baseline score below the median; high functioning = baseline score above the median.

benefit could be through reduction of brain inflammation and may be due to inhibition of different inflammatory processes (many of which involve mast cells), because a subgroup of subjects with ASD present with some immune dysfunction.[16]

Both raw and age-equivalent scores (except for the communication raw score) were greater at the end of the study, indicating an increase in adaptive functioning in all domains (which also was indicated by the increase in the composite score), with the less sensitive standard scores reaching significance only for the social domain. These gains were also clinically significant, as demonstrated by the medium effect sizes. The participants established a growth momentum equal or even slightly greater than the normal development, as shown by the gains in months in all domains and the positive ABI scores. The latter were significantly higher than expected for children of the same age without specific intervention, as reported by Tager-Flusberg (ABIs, −0.20 to −4.6%).[37]

Given the fact that adaptive skills in children with autism tend to improve with time,[39,40] but the progress does not keep pace with their neurotypical peers[39] and tends to slow down as they grow older [41] (with their standard scores declining),[42] we can assume that our encouraging results can be attributed to the study formulation. Although all subjects were receiving behaioral intervention, which is positively correlated with adaptive gains,[39] there was no significant change in the hours received during the study to account for the acceleration in their growth rate nor could we find a correlation between improvement (as measured by using the CGI-I) and at least the hours (but not the quality) of intervention received. The inability to detect significant correlations between differences in VABS raw scores and differences in the ABC subscale scores also indicates that the behavioral improvement observed cannot explain the substantial adaptive functioning gains, and we thus consider them simply a secondary effect. The exception of the ABC social withdrawal score being correlated with the VABS social raw score was to be expected because both of these subscales address the same behavioral parameter.

The observed gains were greater than those reported by the Research Units on Pediatric Psychopharmacology (RUPP) with the use of risperidone [37] or the enrollment of children in specialized autism primary schools and units.[43] The gains were similar to those reported from a 20-month intensive educational program for preschool-aged children.[41] The latter 2 samples were more similar to ours than the RUPP sample, in which subjects were chosen for having more behavioral problems [37] but, at the same time, were younger than our study subjects by 2 and 4 years, respectively; this difference makes them more likely than our sample to exhibit such improvements.[41] Its noteworthy, however, that our sample consisted of a mixed population, comprising subjects enrolled in special autism units as

Table IV. Adverse events reported.

| Subject | Adverse Events |
|---|---|
| 1 | Disoriented, looked lost for a few days |
| | Logy movements 2 weeks after formulation onset that lasted 2 months |
| 2 | A. Abdominal pain 10 to 15 times, of 10 to 60 minutes duration, for 20 days after formulation onset and throughout the study |
| | B. Increased preexisting atypical body movements |
| 3 | Increased frequency of urination for 2 weeks |
| 4 | A. Sleeping difficulties for 13 weeks |
| | B. Increased appetite for 2 months but without weight gain |
| 5 | Development of facial tic 2 months after onset |
| 6 | Increased aggression toward others for the first 4 months |
| 7 | A mild facial rush after the afternoon capsule for 1 hour for the first 2 months |
| 8 | Occurrence of incomprehensible speech along with his irritability, albeit his overall gains in speech |
| 9 | Sleeping difficulties for 2 weeks |
| 10 | Mild abdominal pain 3 months after the formulation initiation and after 2 incidents of gastroenteritis that lasted for 1 month |
| 11 | A. Unjustified occasional laughter |
| | B. Self-spinning 3 to 4 days after the formulation initiation for 2 weeks |
| 12 | Sleeping difficulties for the first 3 days after supplement onset |

well as children well integrated into mainstream schools.

The secondary behavioral gains as shown by ABC subscales were also significant both statistically and clinically, with adequate effect sizes. The fact that the time effect was stronger for week 26 indicates that shorter administration of the formulation will result in less gains. The gains were lower (26.6%–34.8% reduction) than those reported from the risperidone study (45.7%–56.9% reduction)[44] in all subscales except that for inappropriate speech (26.9% vs 8.64% reduction). However, the RUPP sample was composed of subjects with many more behavioral difficulties (eg, mean irritability score of 26.2 vs 13.16; mean stereotypies score of 10.6 vs 5.94), and these difficulties (specifically the irritability reduction) were the main treatment goals. However, our study formulation showed efficacy in alleviating those symptoms as well.

Because ATEC subscales of communication and sociability were reported to correlate significantly with the relevant subscales of the VABS,[45] we expected them to follow the same pattern in change over time. However, only the behavioral ATEC subscale derived significant results at end point. The ATEC includes both developmental and symptom severity items, lacks evidence for its factor structure and item allocation in the subscales, and its scores are substantially influenced by the cognitive level and communication abilities of the subjects.[43,45] These factors could explain this discrepancy in both our findings and those of others researchers.

CGI-I scores indicated only a minor improvement; however, 37.5% of the subjects were assessed as much improved or very much improved. Although the primary clinician gathered information from both the parents and the subjects' trainers in most cases, the weaknesses of this instrument (raters' memory issues, prospective nature and subjectivity, its ambiguous format with no operationalized definitions, the reporting of irrelevant adverse events, and inconsistency between clinicians and the subjects or his or her caretaker's view)[46,47] could account for this finding.

The examination of the baseline moderators[48] of the main outcomes revealed that only the initial level of functioning was predictive of the response to the study formulation, with age, sex, and history of allergies not being significant. The similar results for the secondary measures (ABC and ATEC) may also mean that chil-

dren with more behavioral difficulties could be better responders to the agent.

More benefits were reported from the parents, especially in the cooperation, concentration, communication, and speech domains, with the latter 2 domains also reflected in the VABS communication scale. It is worth noting that although initial transient irritability is the formulation's main adverse effect, the ABC irritability subscale showed a significant improvement at the end of the study. This adverse effect may be related to "intolerance to phenols"; thus, a new formulation with reduced phenol content† is now available.

The results presented here are obviously limited by the fact that the study was not double-blinded or placebo controlled. Furthermore, the instruments chosen may have not captured all the facets of the formulation's impact on ASD children. It seemed that a capsule formulation is not a successful way to administer the flavonoids, as >40% of the participants were unable to swallow the softgel capsules and parents had to open the capsule to administer its contents. This delivery system probably resulted in some subjects receiving a dose lower than what was intended. This administration mode could also account for the 2 children who withdrew as a result of the parents' inability to administer the formulation. Thus, an oral solution of the formulation would be more helpful for this population, even though softgel capsules would be easier to blind in a double-blind study.

## CONCLUSIONS

The current results are encouraging in that the dietary supplement formulation studied here, containing luteolin and quercetin,[49,50] could provide significant benefit in ASD children both in adaptive functioning and behavioral difficulties. The response to the formulation is probably more favourable (ie, more gains are expected) for higher functioning children of all ages with more behavioral problems (stereotypies, hyperactivity, language, and communication problems). Quercetin and luteolin are generally considered safe, and the only adverse effect noted in our subjects was transient irritability.

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## CONFLICTS OF INTEREST

The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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†Trademark: NeuroProtek LP® (Algonot, LLC).

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