ORIGINAL ARTICLE

Behavioral Benefits of Camel Milk in Subjects with Autism Spectrum Disorder

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Abstract

Objective: To investigate the possible therapeutic effects of camel milk on behavioral characteristics as an interventional strategy in autistic children.

Study Design: Double-blind, Randomized Clinical Trial (RCT).

Place and Duration of Study: Autism Research and Treatment Center, Al-Amodi Autism Research Chair, Department of Physiology, Faculty of Medicine, King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia, from October 2012 to May 2013.

Methodology: Changes in behavioral characteristics in 65 (boys=60, girls=5) children with autism (aged from 2 to 12 years) were assessed. The behavioral symptoms were evaluated by Childhood Autism Rating Scale (CARS), Social Responsiveness Scale (SRS), and Autism Treatment Evaluation Checklist (ATEC) before and after the 2 weeks of camel milk therapy.

Results: Significant differences were detected on Autism Spectrum Disorder (ASD) by CARS, SRS and ATEC scales, following 2 weeks of camel milk consumption, but not in the placebo group.

Conclusion: The present study demonstrates that camel milk could be very promising therapeutic intervention in ASD. Further wide scale studies are strongly recommended.

Key Words: *Autism spectrum disorder (ASD). Camel milk. Behavioral symptoms.*

INTRODUCTION

Autism Spectrum Disorders (ASDs) are a wide range of neurodevelopmental conditions that demonstrate considerable phenotypic heterogeneity, both in terms of presentation at any one age and across development. The current classification systems include three domains of difficulties: reciprocal social interaction, abnormalities in communication, and patterns of nonfunctional restricted, repetitive and stereotyped behaviors.[1] Although there is no known unique cause of autism, there is growing evidence that autism can be caused by a variety of factors including autoimmunity[2] originated by dairy food allergy. While several intervention methods for ASDs have been used to treat children with autism spectrum disorders, very few have been subjected to careful scientific investigation.

Autoimmunity to CNS was also documented by several research groups, through the presence of brain specific auto-antibodies in the brains of some autistic children.[3] The reason behind the formation of some brain auto-antibodies in some patients with autism is not fully understood. It is speculated that an autoimmune reaction to neurons might be triggered by some cross-reacting antigens in the environment resulting in the release of neuronal antigens. These neuronal antigens may result in induction of autoimmune reactions through the activation of the inflammatory cells in genetically susceptible individuals. The environmental antigens may include food allergies to certain peptides such as gliadin, cow's milk protein and soy.[4]

Camel milk has emerged to have potential therapeutic effects in diseases such as diabetes,[5] and hepatitis B,[6] as well as cow milk allergy in children.[4] Children with severe food allergies improved rapidly with camel milk.[7] It contains various 'protective proteins' (Lysozymes, Immunoglobulins, Lactoferrin, Lactoperoxidase, Peptidoglycan Recognition Protein (PGRP) and N-acetyl-§-glucosaminidase (NAGase); mainly enzymes which have antiviral, antibacterial and immunological properties.[7]

Recently, Bashir and Al-Ayadhi investigated[8] the role of the effectiveness of camel milk (raw and boiled) on Thymus and Activation-Regulated Chemokine (TARC) serum levels and CARS score in subjects with autism and compared to placebo group (cow milk). The results suggested that camel milk therapy over the course of two weeks, significantly decreases the serum levels of TARC among the study subjects and also improve clinical measurements of ASD severity (CARS score).

The above findings initiated the authors' interest to test the effectiveness of camel milk on behavioral changes in

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subjects with Autism Spectrum Disorders. The hypothesis tested in the present study was that autism can be caused by food allergy.[9] It has been believed that normal diary food is harmful to the immune system, brain and bodies of children with ASD[10] and have a significant impact on behavior, cognition, socialization, and health/physical traits associated with an ASD diagnosis. The present prospective, double-blind, placebo controlled trial has evaluated whether a standardized treatment of camel milk administered to patients diagnosed with ASD on a daily basis for 2 weeks would result in improved behavior, cognition, socialization, and health/physical traits associated with an ASD diagnosis.

METHODOLOGY

The study was a double-blinded, Randomized Clinical Trial (RCT) conducted at Autism Research and Treatment Center, Al-Amodi Autism Research Chair, Department of Physiology, Faculty of Medicine, King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia, from October 2012 to May 2013.

Autistic children with typical symptoms, especially those with known allergies or food intolerances, were randomly recruited in this study. The patients were referred by neuropediatric clinics from all around the Kingdom of Saudi Arabia. This study protocol received the ethical approval from the Institutional Review Board of King Saud University, Faculty of Medicine. Participants were given a complete description of the study and a written informed consent was obtained from all parents/guardians before they were enrolled in the study.

The inclusion criterion for the autism group was meeting the cut-off score of the Autistic Disorder based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria.[1] All participants were screened via parental interview for current and past physical illnesses. Subjects were excluded from the investigation, if they had dysmorphic features or diagnosis of fragile x or other serious neurological (e.g., seizures), psychiatric (e.g., bipolar disorder) or known medical conditions. Children with known endocrine, cardiovascular, pulmonary, and liver or kidney disease were also excluded from the study.

The participants were randomly divided into three groups: Group I (n=25) received pasteurized camel milk; Group II unpasteurized camel milk (n=22) and Group III received cow milk as a placebo (n= 18). All groups received the same instructions, volume of milk and containers to preserve the blinding of the study.

Parents were instructed to include an average of 500 ml of camel milk in their children's regular daily diet for a period of 2 weeks. Parents were asked to continue with the children's daily routines. They were not allowed to add or remove any interventions such as diet plans, supplements or pharmacotherapies throughout the study period. It was also instructed to drink cold milk, beginning with small quantities and increase gradually until 500 ml per day to avoid any risk of diarrhea.

ARTC psychologist assessed the disease severity through baseline psychology scales including Childhood Autism Rating Scale (CARS),[11] Social Responsiveness Scale (SRS)[12] and Autism Treatment Evaluation Checklist (ATEC).[13]

Medical history of the child and family was obtained through a structured questionnaire interview conducted with the parents/legal guardians. Height and weight of the patients were recorded. During the study period, the patients' progress were monitored by phone calls. After 2 weeks, the participants returned for a follow-up where all psychology scales were conducted. All observations by parents were also noted. Safety evaluations including physical examinations were carried out by the prime investigator for patients who showed any negative symptoms.

Fresh camel milk was obtained by ARTC from a trusted camel farm who ran regular routine veterinary checkups on the camels. After receiving the milk, microbiological screening tests were conducted on all milk batches to ensure that it was free of pathogens commonly found in raw camel milk.[14] The pathological screenings were conducted to detect Campylobacter, Bacillus cereus enterotoxin, *E. coli* O157:H7, Listeria, Salmonella by GLISA rapid testing using the kits Singlepath Campylobacter, Doupath Cereus Enterotoxin (EMD chemicals), Reveal *E. coli* O157:H7, Salmonella, Listeria (Neogen) and B. Brucela (Anigen). Any batch tested positive for the above mentioned pathogens was immediately excluded from the above study. Camel milk supplied to Group I was pasteurized by heating to 65°C for 15 seconds, then removed, cooled in a ice pot initially and then stored in the freezer at -80°C. Milk supplied to Group II was not heated to avoid losing beneficial nutrients and proteins.[15] Frozen milk was supplied to patients using BPA-free freezer bottles and thawed on countertops as needed.

The data were prospectively collected, analyzed and results were presented as mean ± SD (standard deviation). Statistical differences in scores in each scale CARS, SRS, and ATEC before and 2 weeks after milk therapy were determined by means of paired sample t-test with $p \leq 0.05$ considered as significant.

RESULTS

There were a total of 65 children including 60 males and 5 females. Changes in behavioral characteristics in 65 (n=65) subjects with autism aged 2 - 12 years, mean = 7.8 years, were assessed. The behavioral symptoms were evaluated before and after 2 weeks of milk consumption period, by the CARS, SRS and ATEC in three groups of autistic children having a clinical

diagnosis by (DSM-IV).[1] The lower the scores are, the less severe the symptoms are. Changes in all measures of symptoms of autism over the course of the study are shown in Tables I - III.

Table I shows the CARS evaluations scoring system which illustrates statistically significant changes in the raw camel milk group (mean score before = 37.6 ±6.3, after = 34.5 ±5.2, p=0.004), and the boiled camel milk group (mean score before =37.1 ±3.6, after = 33.8 ±4.9, p=0.0001). Furthermore, there were no significant changes in the placebo group (mean score before = 34.2 ±3.3, after = 33.8 ±3.5, p=0.41). These changes represented reductions of 8% and 9% in each raw and boiled camel milk . However, no significant change was observed in cow milk as far as CARS score is concerned.

SRS mean score for all groups along with each subscale was calculated (Table II). The SRS evaluations showed statistically significant changes in mean SRS subscale scores in social cognition (p=0.002), social communication (p=0.018) and social awareness (p=0.050), for the raw camel milk group. On the other hand, the boiled camel milk group demonstrated a significant change only in social cognitions subcategory (p= 0.0001). No significant change in placebo group was observed.

The ATEC (mean ± SD) scores of different categories of camel milk (raw and boiled) and placebo groups are shown in Table III. The ATEC evaluations showed that ATEC total and subscale scores in different categories do not show significant changes in camel milk groups (raw and boiled) compared to the placebo group except speech/language/communication in boiled camel milk group (p=0.0001).

Table I: Change in clinical outcome measures in CARS scores in autistic children.

	Score before therapy Mean ± SD	Score after therapy Mean ± SD	p-value
Raw camel milk	37.6 ± 6.3	34.5 ± 5.2	0.004*
Boiled camel milk	37.1 ± 3.6	33.8 ± 4.9	0.0001*
Placebo (cow milk)	34.2 ± 3.3	33.8 ± 3.5	0.41

**significant*

DISCUSSION

Upto date there is no known effective approved intervention method for autism spectrum disorders. Consequently, this creates many challenging issues and it has become an area of a major controversy. Over the last few years, a number of research groups suggested possible autoimmunity as a significant etiological factor in autism.[16]

This study represents the first prospective study on the use of camel milk as potential therapeutic intervention strategy for children with autism. In this study, camel milk (both boiled and raw) demonstrated significant effect on some autistic behaviors, through improvement in social cognition, social communication, and social awareness (SRS). Furthermore, boiled camel milk produced significant improvement in speech/language/ communication (ATEC). This was supported by the significant changes in the CARS scoring results. Camel milk with its unique characters could be a promising therapeutic intervention strategy in autism spectrum disorders.

Table II: Change in clinical outcome measures in SRS scores in the autistic children.

Sub Scales	Raw camel milk			Boiled camel milk			Placebo (cow milk)		
	Score before therapy Mean ± (SD)	Score after therapy Mean ± (SD)	p-value	Score before therapy Mean ± (SD)	Score after therapy Mean ± (SD)	p-value	Score before therapy Mean ± (SD)	Score after therapy Mean ± (SD)	p-value
Social cognition	75 (4.3)	70 (5.3)	0.002*	75 (3.8)	70 (4.1)	0.0001*	78 (7.2)	72 (7.5)	0.437
Social communication	72 (5.7)	70 (6.6)	0.018*	71 (5.5)	68 (5.5)	0.076	75 (9.0)	73 (5.3)	0.722
Social motivation	74 (6.5)	73 (4.0)	0.301	73 (4.9)	72 (19)	0.482	75 (6.1)	74 (5.1)	0.225
Autistic mannerism	78 (7.1)	78 (7.4)	0.219	78 (6.4)	75 (20)	0.073	82 (8.0)	79 (9.8)	0.423
Social awareness	71 (5.4)	70 (5.4)	0.050*	72 (5.1)	72 (5.8)	0.650	79 (8.3)	67 (5.0)	0.113
Average	75 (6.2)	74 (6.3)	0.037*	75 (5.0)	73 (5.0)	0.033*	81 (8.5)	75 (8.1)	0.104

**significant*

Table III: Change in clinical outcome measures in ATEC subscales scores in autistic children.

Sub Scales	Raw camel milk			Boiled camel milk			Placebo (cow milk)		
	Score before therapy Mean ± (SD)	Score after therapy Mean ± (SD)	p-value	Score before therapy Mean ± (SD)	Score after therapy Mean ± (SD)	p-value	Score before therapy Mean ± (SD)	Score after therapy Mean ± (SD)	p-value
Speech/language/ communication	4.6 (6.1)	3.5 (3.0)	0.200	7.6 (7.9)	6.2 (6.8)	0.012*	10 (7.0)	12 (8.4)	0.548
Sociability	23 (6.3)	21 (3.7)	0.061	21 (6.4)	21 (8.4)	0.790	23 (7.9)	22 (4.9)	0.430
Sensory/cognition/ awareness	24 (7.3)	21 (5.5)	0.131	23 (8.8)	21 (12)	0.132	24 (8.3)	24 (6.6)	0.838
Health/physical/ behavior	24 (7.3)	22 (5.2)	0.120	21 (9.1)	20 (6.1)	0.405	24 (8.4)	23 (5.4)	0.421
Total	74 (15)	72 (10)	0.566	70 (16)	69 (18)	0.838	80 (14)	76 (13)	0.156

**significant*

Since recent reports demonstrated higher oxidative stress statues in ASD subjects compared to normally developing controls[17], it makes camel milk an ideal antioxidant food. Furthermore, camel milk can certainly play important role in the prevention of diary food allergies and has been used to treat children with autism.[7] However, to date; few studies reported some improvements in symptom scores in children who were treated with camel milk.[18,19]

A significant therapeutic effect of raw camel milk is decreased on boiling even on pasteurization.[20] Camel milk has good bacterial and anti-viral activity thus if is used raw, there are less chances of transmission of infection. This concept is consistent with the historic belief that natural substances play an important role in preventative and therapeutic treatment.[20]

Milk protein casein plays important role in the food allergies related disorders and cause autism.[18] Many children with autism may have gastrointestinal difficulties that make it hard for them to digest milk protein properly. There are different possibilities for ways in which this could affect children with autism. This could be through the unique immunological properties of camel milk immunoglobulins (Igs) including unique subclasses IgG2 and IgG3, contribute to camel milk's incredible infection fighting and eradication capacity. Camel Igs being so small are able to penetrate into tissues and cells to completely neutralize the enzyme activity of an infectious agent such as a bacteria or virus whereas, human antibodies Igs cannot.[21] Second possibility, is through the strong antioxidant properties of camel milk.[22] Bioactive peptides derived from camel milk protein showed higher functionality including antioxidant activity, anti-hypertension effect and antimicrobial activity comparing to bioactive peptides from bovine milk proteins.[23] Last but not the least, it is a fact that camel milk does not contain allergens like beta-lactoglobulin and a “new” beta-casein which are present in cow milk and thus makes the camel milk attractive for children suffering from milk allergies.[7] Another relevant fact is that the components of camel milk include immuno-globulins similar to those in mothers' milk, which reduce children's allergic reactions and strengthen their future response to foods. The beta-casein in camel milk is completely a different protein due to the amphipathic structure; so it has a strong inherent tendency to self-associate into micelles of 15 - 60 molecules. Association and conformational changes can have a major influence on the function of beta-caseins.[24]

Casein molecules are actually micelles and camel milk micelles have been found to be larger in size (15 nm) than those of cow milk or human milk. Camel milk has a lower pH than other milk, so upon entering the stomach the casein micelles do not breakdown into casein and, therefore, do not break into casomorphins. Casomorphin creation from cow milk consumption is a common problem in autism that increases autistic symptoms.[25]

Further studies are needed by other investigators to confirm these findings; however, in the light of the positive results of this study and those of several previous studies,[18,19] the use of camel milk appears to be a promising treatment for children with autism. Camel milk therapy was safe and well-tolerated. None worsened and no side effects were reported.

CONCLUSION

Autism is a severe, lifelong disorder with serious emotional and financial consequences. Its incidence is rapidly increasing, and its etiology is still unclear. The present study demonstrates that camel milk could be very promising therapeutic intervention in ASD. Further wide-scale studies are strongly recommended.

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Effect of camel milk on thymus and activation-regulated chemokine in autistic children: double-blind study

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BACKGROUND: This study aimed to investigate the role of the effectiveness of camel milk (CM) (raw and boiled) on thymus and activation-regulated chemokine (TARC) serum levels and childhood autism rating scale (CARS) score in subjects with autism and compared to placebo group (cow milk).

METHODS: Forty-five subjects diagnosed with autism were randomly assigned to receive boiled CM for group I ($n = 15$), raw CM for group II ($n = 15$), and placebo for group III ($n = 15$) for 2 wk. Measures included changes in professionally completed CARS score and blood samples for TARC serum level were taken before and after milk consumption of 500 ml per day in children's regular daily diet.

RESULTS: The serum levels of TARC decreased significantly ($P = 0.004$) in boiled CM and in raw CM group ($P = 0.01$) too, but no effect was observed ($P = 0.68$) in placebo group. Furthermore, significant improvements were observed in CARS score ($P = 0.04$) in raw CM group only. There were no significant relationships between the serum of TARC level and the CARS score, age, or gender for any group.

CONCLUSION: CM administered for 2 wk significantly improved clinical measurements of autism severity and decreased serum level of TARC in autistic children, but subsequent studies are recommended.

Autism is a severe neurodevelopmental disorder that is characterized by impairment in verbal and nonverbal communication, imagination, reciprocal social interaction, and evidence of developmental delay within the first 3 y of life (1–4). Immunological and environmental factors, such as diet, infection, and xenobiotics play critical roles in the development of autism (4–6). Over the years, research findings, especially from our lab, suggested possible involvement of altered immune system in the pathophysiology of autism spectrum disorder (ASD) (7,8). Despite the clear unmet medical need, currently, there is no recognized effective comprehensive treatment (9).

Proinflammatory chemokines, such as monocyte chemotactic protein-1 and thymus and activation-regulated chemokine (TARC), along with cytokines, such as tumor necrosis factor α, were consistently elevated in the brains of individuals with autism (7,8). At critical times of infantile development, immune dysregulation may result in the release of immune-modulatory molecules, such as chemokines and cytokines, leading to altered neuronal development and neural function (10,11). Furthermore, Ashwood and colleagues (2008) found that reduced levels of the modulatory cytokine, transforming growth factor-β1 (TGF-β1), in autistic children contributed to the dysregulation of adaptive behaviors and predisposal for autoimmune responses (12).

Milk is an important nutrient in human nourishment. In some communities, camels represent the most important source of this nutrient. Camel milk (CM) has emerged to have potential therapeutic effects in diseases such as diabetes (13,14), hepatitis B (15), possibly certain symptoms accompanying autism enterocolitis, H. pylori infection and lactase deficiency might be cured with CM as well (16). Recently, some parents have been using camels' milk as a treatment in some children with ASD because camels' milk appears to help food allergies in some individuals (17,18). CM's lactoferrin has very high levels of bactericidal and bacteriostatic properties against Gram-positive and Gram-negative bacteria (19), more than cow and human lactoferrin. CM contains various protective proteins, mainly enzymes which exert antibacterial and immunological properties (20). The fact that CM lacks β-lactoglobulin and a "new" β-casein (21), two powerful allergens in cow milk, makes the milk attractive for children suffering from milk allergies (22). Phylogenetic differences could be responsible for the failed recognition of camels' proteins by circulating IgEs and monoclonal antibodies (23). Children with severe food allergies improved rapidly with CM. CM with its unique properties, such as, high vitamin C levels, low fat content, and low molecular weight immunoglobulins, makes an ideal natural intervention method in ASD.

The hypothesis tested in the present study was that TARC act on their chemokine receptor type 4 (CCR4) receptors to enhance the recruitment and activation of T helper 2 cells with a subsequent production of type 2 cytokines that include interleukin (IL)-4, IL-5, IL-9, and IL-13 (24,25). CCR4 ligands have an important pathogenic role in inflammatory conditions such

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as allergy and some autoimmune diseases (26–28). TARC and their receptors have been implicated as functional mediators of immunopathology of autoimmune neuroinflammatory diseases (29,30) and childhood autism rating scale (CARS) score which have a significant impact on behavior, cognition, socialization, and health/physical traits associated with an ASD diagnosis.

The present prospective, double-blind, placebo-controlled trial evaluated whether a standardized CM administered to patients diagnosed with an ASD on a daily basis for 2 wk would result in improved CARS score traits associated with an ASD diagnosis.

RESULTS

The general characteristics of the study participants and the results of the serum levels of TARC are depicted in **Table 1**. No significant differences were observed between the CM (raw or boiled) and placebo groups with respect to age, gender, or ASD diagnostic status. There were no significant differences on serum levels of TARC at baseline between the randomization groups ($P = 0.28$) and on the CARS score between groups ($P = 0.719$).

Figure 1 summarizes the change in serum levels of TARC after the CM (raw or boiled) and placebo groups following 2 wk of therapy. Changes in serum levels of TARC significantly decreased ($P = 0.004$, $P = 0.01$, for values see **Table 2**) for CM (boiled and raw) group respectively. In contrast, no similar changes were observed ($P = 0.54$, for values see **Table 2**) for placebo group. There was a significant difference ($P = 0.04$, for values see **Table 3**) for CARS score from 37.13 ± 5.3 (mean ± SEM) to 33.8 ± 2.7 (mean ± SEM) in raw CM group only. No significant correlation was found between serum TARC levels and CARS score for children with ASD for any group.

Table 1. Baseline characteristics of camel milk group (raw and boiled) and placebo (cow milk) cohorts at randomization

	Camel milk group		
	Raw	Boiled	Placebo group
Age (year)	7.1 ± 3.8[a]	6.8 ± 4.1	6.9 ± 4.3
Gender (*n*)			
Male	13	14	13
Female	2	1	2

[a]Mean ± SD.

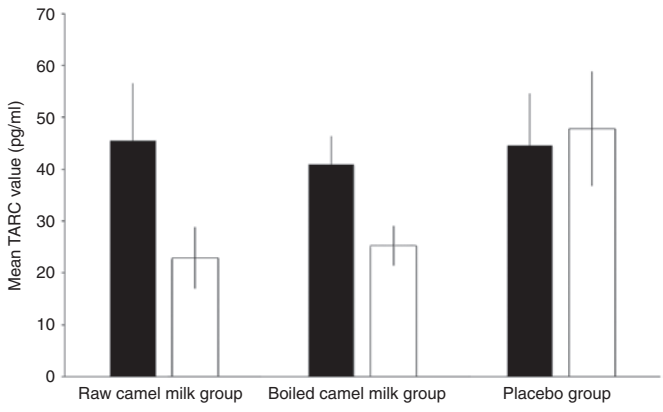

Figure 1. Serum levels of thymus and activation-regulated chemokine (TARC) before (black columns) and after (white columns) in camel milk group (raw and boiled) and placebo (cow milk) group of autistic children.

DISCUSSION

The present study is the first prospective, double-blind, placebo-controlled trial to evaluate the effects of CM therapy among subjects diagnosed with an ASD. In the present study, CM therapy (raw CM group) for 2 wk among subjects diagnosed with an ASD significantly improved clinical measurements (CARS score) recorded by trained professional and parents of study subjects. Furthermore, CM therapy (raw and boiled) significantly decreased serum levels of TARC among patients diagnosed with an ASD. Finally, CM therapy was generally well tolerated with minimal adverse effects. The side effects in the children who did not tolerate the treatment well were irritability and/or stomach discomfort.

CM, with its distinctive properties, could be a promising beneficial intervention approach in ASD. Mammalian antibodies composed of two identical H-chains and two identical L-chains (31). Camel IgG antibodies are heavy-chain antibodies that lack the L-chain (31). The size of the antibodies is a major issue in the development of human immunotherapy. Camel antibodies are only one tenth of the size of human antibodies, which makes them natural nano-bodies (31,32). Furthermore, the high content of vitamin C in CM gives it a string antioxidant property (33). Recent reports have demonstrated higher oxidative stress status in ASD subjects compared to normally developing controls (34–36), which makes CM an ideal antioxidant food for ASD subjects. In addition, those unique properties of CM most probably reduced TARC synthesis and secretion, and consequently, reducing the neuroimflammation and the autoimmune reaction, leading to improved behavior, reflected on improved CARS scoring results.

Table 2. Serum levels of TARC results for camel milk group (raw, boiled) and placebo (cow milk) groups

	TARC (pg/ml)	
	Before	After
Boiled camel milk group (*n* = 15)	40.09 ± 5.4[a]	25.25 ± 3.08*
Raw camel milk group (*n* = 15)	45.52 ± 11	22.86 ± 5.9*
Placebo group (*n* = 15)	44.46 ± 10.11	47.8 ± 11

TARC, thymus activation-regulated chemokine.
[a]Mean ± SD. *Significance level ($P = 0.05$).

Table 3. Childhood autism rating scale scoring results for camel milk (raw, boiled) and placebo (cow milk) groups

	Before	After
Boiled camel milk group (*n* = 15)	38 ± 5.4[a]	35 ± 2.7
Raw camel milk group (*n* = 15)	37 ± 3.8	32 ± 2.6*
Placebo group (*n* = 15)	36 ± 3	33 ± 3.4

[a]Mean ± SD. *Significance level ($P = 0.05$).

However, a clear pattern has emerged over several studies that shows altered levels of immune mediators are associated with increased impairments in behaviors (7,12,37,38) and suggests that a dysregulated immune response is related to behavioral and cognitive impairments in children with ASD. The elements such as zinc, copper, selenium, and iron are not likely to have influenced our results, since their amounts in CM and in cow milk are practically the same (39).

Strengths/Limitations

The main strength of the present study is the design as a prospective, double-blind, placebo-controlled trial. Every effort was made to ensure that the present study was truly double-blind so that those evaluating study subjects, both trained professionals and parents, had no knowledge as to the treatment status of any particular study subject. Furthermore, the present study also attempted to minimize the effects of study drop-out for potential adverse reactions in the data, especially for CARS scores. These particular scoring measurements were conducted by the study investigators on each child regardless of whether or not they dropped-out from the study for potential adverse reactions. For each outcome measurement evaluated, the relative change for the parameter following 2 wk of therapy in comparison to baseline was examined. As a result, potential variation between study subjects was minimized because each study subject served as his or her own control.

One of the potential limitations of the present study is the small sample size examined. The small sample size in the present study may have resulted in specific effects of CM therapy being missed because of lack of statistical power to detect significant changes between the CM (raw or boiled) and placebo groups. As a result, the observation of significant positive effects of CM therapy in the present study tends to argue that the observed effects represent genuine phenomena. The data from the present study provide the basis for a larger, more focused study on the promising elements.

A further potential limitation of the present study is the exact mechanism of action of CM was not elucidated from the present study. Finally, an additional potential limitation of the present study is the fact that the dose of CM used may not have been optimal. The dosing regimen of CM used in the present study was derived from pediatric nutrition, as the recommended starting dose for children.

Conclusion

Camels' immune systems are stronger than that of humans and the small immunoglobulins pass from the CM into the human blood. As immunoglobulins are found in CM throughout lactation, drinking milk will provide a "tool" for combating autoimmune diseases by rehabilitating the immune system rather than is depression.

In conclusion, the results of the present study suggest that CM therapy over the course of 2 wk of therapy significantly improved clinical measurements of ASD severity (CARS score). Furthermore, there were significant decrease levels of serum of TARC among the study subjects examined. Overall, the CM therapy was well tolerated. It is suggested that future studies further explore the biological basis for CM's mode of action at the cellular level in those patients diagnosed with an ASD who would most benefit from CM therapy.

METHODS

Subjects

A total of 45 subjects diagnosed with ASD, aged from 2 to 12 y (40 males, 5 females), were recruited to the study. The study subjects have body weight between 12.8 kg and 42.6 kg. None of the study subjects had previously received CM therapy. None of the study subjects had any change in therapy or treatment (including medications) within 1 mo prior to the study. Patients fulfilled the criteria for the diagnosis of autism according to the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (2). The study protocol received Institutional Review Board approval from King Khalid Hospital (King Saud University, Riyadh, Saudi Arabia). All parents signed a consent form and all received a copy.

Clinical Assessment

Autism Diagnostic Observation Schedule is a semistructured, standardized observational instrument to assess the social and communicative abilities of individuals with possible ASD. Items are scored from 0 (not abnormal) to 2 or 3 (most abnormal), and a diagnosis of autism or ASD is established if the individual assessed has scores higher than the established cut-off values in the communication domain, the social domain, and a sum of the two (40).

Clinical Measure

Childhood autism rating scale. Study participants were evaluated using CARS test conducted only by a single child psychiatrist who observed the subjects and interviewed the parent(s) and was unaware as to the treatment status of the subject. The CARS test is a 15-item behavioral rating scale (relating to people, emotional response, imitation, body use, object use, listening response, fear or nervousness, verbal communication, nonverbal communication, activity level, consistency of intellectual response, adaptation to change, taste, touch and smell response, and general impressions) developed to identify autism as well as to quantitatively describe the severity of the disorder. The CARS test is a well-established measure of autism severity (41). The internal consistency reliability alpha coefficient is 0.94; the interrater reliability correlation coefficient is 0.71; and the test-retest correlation coefficient is 0.88 (42). CARS scores have high criterion-related validity when compared to clinical ratings during the same diagnostic sessions, with a significant correlation of 0.84 (42).

Lab Testing

Blood samples. After an overnight fasting, blood samples (3 ml) were collected from subjects in both groups in plain test tubes. Blood samples were allowed to clot and then centrifuged at 3,000 rpm to collect serum samples, which were stored frozen in a freezer at −80 °C until the time of analytical assays. The detail procedure has been described in our previous work (7,8,43)

Chemokine assay. Serum level of TARC was measured using a commercially available sandwich enzyme immunoassay (ELISA) kit from CUSABIO BIOTECH (Wuhan, China).

Study Milk

CM (boiled or raw) was supplied in a liquid preparation. The placebo (cow milk) group was identical in appearance. The recommended childhood starting dose of 500 ml milk per day (half the total dose administered in the morning and half the total dose administered in the evening). Study subject–specific dosing instructions were placed on each liquid preparation provided to study subjects. Parents were asked to continue with the children's daily routines. They were not allowed to add or remove any interventions such as diet plans, supplements, or pharmacotherapies throughout the study period.

Milk handling. Fresh CM was obtained from a trusted camel farm that ran regular routine veterinary checkups on the camels. After receiving the milk, microbiological screening tests were conducted on all milk batches to ensure that it was free of pathogens commonly found in raw CM (44). The pathological screenings were conducted to detect *Campylobacter* (KGaA, Darmstadt, Germany) *Bacillus cereus* enterotoxin, *E. coli* O157:H7, *Listeria, Salmonella* by GLISA rapid testing using the kits Singlepath *Campylobacter*, Duopath Cereus Enterotoxin (EMD chemicals), Reveal *E. coli* O157:H7, *Salmonella, Listeria* (Neogen), and *B. Brucella* (Anigen). Any batch-tested positive for the mentioned pathogens was immediately excluded from the study. CM supplied to group I was pasteurized by heating to 65 °C for 15 s, then removed, cooled in a ice pot initially and then stored in the freezer at −80 °C. Milk supplied to group II was not heated to avoid losing beneficial nutrients and proteins (45). Frozen milk was supplied to patients using bisphenol-A-free freezer bottles and thawed on countertops as needed.

Study Design

This was a randomized, double-blind, placebo-controlled study. The study was conducted between 2011 and 2012. The study subjects were recruited through community contacts. The study protocol called for 36 subjects to receive CM (raw or boiled) and 18 study subjects to receive placebo (cow milk). A total of 54 subjects were recruited for the present study. Four subjects withdrew prior to randomization into CM or placebo groups. A total of 54 subjects were randomly assigned to receive CM (boiled or raw) or placebo, and of these, a total of 9 subjects (4 in boiled, 2 in raw in CM group and 3 in the placebo group) withdrew prior to successful completion of 2 wk of therapy. Among the nine subjects withdrawing from the study prior to successful completion of 2 wk of therapy, four subjects withdrew because of adverse reactions (two in raw CM group, one in boiled CM group and 1 in the placebo group), three subjects did not comply with the study protocol, and two was lost to follow-up with no known adverse reaction. In addition, study investigators monitored study subjects to ensure compliance and to monitor for potential adverse reactions.

Prerandomization Phase

Study subjects were seen for an initial screening where study investigators obtained information regarding demographics, formal diagnosis, age at diagnosis, age of apparent onset, information regarding delay or regression, any current medical issues, medications, bodyweight, and allergies on each study subject. A baseline CARS evaluation was performed by a child psychiatrist. In addition, blood samples were collected on each study subject at an autism research and treatment center draw station.

Randomization Phase

Following the initial screening and collection of labs, all study subjects started therapy within 30 d of baseline measurements. A study investigator, who did not perform any clinical measurements on study subjects, used a coin-flip to randomly assign study subjects to either the CM (raw or boiled) or placebo groups. Since there was a difference in sample size between the CM and placebo groups, the placebo group was filled with study subjects before the treatment group, so that the latter study subjects were all assigned to the CM group (raw or boiled). Study investigators in contact with the study subjects and the parents of study subjects were not informed of the treatment status (CM/placebo) of each study participant until all study subjects had completed the trial, and hence the assignment (CM/placebo) strategy used should not have revealed any information regarding the treatment status of any study participant to study investigators in contact with study subjects and the parents of study subjects. For the duration of the trial, any concomitant use of drugs/supplements were not changed as far as possible.

Statistical Analysis

The results were analyzed using the commercially available software package Statview (Abacus concepts, Berkley, CA). The data are presented as the means ± SEM. The Mann–Whitney test was used for comparisons between data. The null hypothesis was that there would be no difference in the data distributions of the relative change in test results following 2 wk of treatment in comparison to baseline measurements between study subjects receiving CM (raw or boiled) in comparison to placebo (cow milk). In addition, the relationship between the change in serum levels of TARC following 2 wk of treatment in comparison to his or her baseline measurements, and the changes in specific outcome measurements (CARS scores) 2 wk of treatment in comparison to his or her baseline measurements. Spearman's rank correlation coefficient "r" was used to determine the relationship between variables. For all statistical tests performed in the present study, a two-tailed P value ≤0.05 was considered to be statistically significant.

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PATIENT CASE REPORT

Patient Report: Autism Spectrum Disorder Treated With Camel Milk

患者报告：使用骆驼奶治疗自闭症谱系障碍

Informe de paciente: trastorno del espectro autista tratado con leche de camella

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Camel milk, autism spectrum disorder, patient report

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Ms Adams completed the ICMJE Disclosure Form for Potential Conflicts of Interest and had no conflicts related to this work to disclose.

BACKGROUND

This patient report is about my son, who was diagnosed with autism spectrum disorder (ASD) at 3 years of age, and the effects I observed when he began drinking camel milk daily. Beginning at age 9, he drank one half cup of raw camel milk a day and experienced overnight an improvement in his symptoms. His continued regular consumption of camel milk was associated with sustained symptom improvements for 6 consecutive years (2007-2013). This patient report is a road map of my navigations, consultations with experts and autism care providers, and the apparent effect of camel milk on autism spectrum disorder (ASD).

Editors' Remarks

In this patient report, a mother shares her observations and assessment of the effectiveness and safety of camel's milk for her autistic son. We believe this patient report helps to communicate her experience of the care her family received. It will also inform clinicians about how patients experience the care they provide. We support reporting the patient's perspective.

INTRODUCTION

As an infant, my son appeared normal and met the generally accepted growth and development milestones. He was calm and attentive, smiled at 6 weeks, laughed, and could focus on books and toys. He was affectionate and bonded with his parents and always showed appropriate separation anxiety. He spoke two clear words at 9 months and walked on his first birthday. However, beginning at 6 months, he started biting people and never pointed to objects. He also had very red cheeks, constipation, prolonged startle reflex, and infant torticollis.

EARLY AUTISM

Just before he turned 3 years old, my son was diagnosed with autism. He had loss of language and attention at 15 to 18 months, the appearance of hyperactivity, sensitivity to noise, and fixation on objects and water. He had difficulty interacting with others, was still biting and engaging in aggressive behavior, and had been dismissed from two preschools. Like many ASD children, he was found to have food intolerances and allergies, skin conditions, auditory processing delay, expressive/receptive language delay, constipation, and an intermittent tic disorder.

After the diagnosis and continuing for years, he received a battery of tests including complete physical exams, electroencephalograms, neurological and sensory evaluations, auditory testing, and stool and urine testing for heavy metals, amino acids, organic acids, intestinal parasites, and *Candida*. Laboratory tests were ordered, including complete blood counts, metabolic profiles, and tests for immune-globulins and inflammatory markers. He also received regular vaccination through 15 months.

My son was enrolled in 35 to 40 hours per week of intensive one-on-one therapy at our home in a clinically supervised program of applied behavioral analysis (ABA). He also had 3 hours per week of individual speech therapy and 2 hours per week of occupational therapy. His diet was gluten- and casein-free for 2 years with limited intake of sugar, yeast, and nuts. His medications included various antiviral (famciclovir, valaciclovir) and antifungal medications (nystatin, ketoconazole, amphotericin-B), selective serotonin reuptake inhibitors (SSRIs; citalopram, escitalopram) and blood pressure–reducing medication (guanfacine) to decrease hyperactivity and aggression. These medications, used daily and mostly added one at a time for careful observation, seemed to be beneficial. The combination of his treatments produced positive benefits that were confirmed in twice-monthly ABA clinic meetings, daily ABA data gathering, and pediatric ASD specialist office visits. As many children make limited gains with similar treatments, this progress earned my son a reputation as a "responder" in autism parlance.

By age 5, this hard-working child had demonstrated significant improvement, passed the kindergarten readiness test, and began attending a public school with a shadow aide. ABA therapy dropped to 3 hours per week. He later attended a school for children with attention deficit/hyperactivity disorder with weekly social skill sessions and participated in lessons and activities outside our home with and without an aide. His schoolwork was at or above grade level with the anticipated exception of hand-

writing. Although he needed intermittent supervision to stay on task, his cognitive scores were all above average. He continued to struggle with prolonged eye contact, and his conversations, while inquisitive and mutually engaging, were sometimes inattentive and monologue-style. Nevertheless, he was bright and friendly and enjoyed social contact and outings with peers and friends. Interestingly, when he ate dairy products around age 7, even pizza with the cheese removed at a baseball game, he would develop many symptoms. Hand-flapping, circle and toe walking, inattention, and constipation would result within hours, and he once complained, "It feels like there's dirt in my brain." Though he had returned to gluten 2 years after his ASD diagnosis with no outward effects, he avoided dairy products and kept his sugar intake low.

Despite this remarkable progress, at age 9, my son began to have significant behavioral issues: sudden hyperactivity, loss of attention, distracted language, and loss of self-regulation. These symptoms were exacerbated when he had not eaten for 2 to 3 hours. Visits to his treating ASD physicians, titration of existing medications, and additional dietary measures did not seem to help. A trial of SSRI (fluoxetine) made him dazed and anxious and seemed to worsen the behavioral issues. It was becoming difficult to prompt and cope with his actions as discipline and safety techniques were no longer effective. As his mother, I was increasingly taxed and my outlook was becoming ominous.

TREATMENT WITH CAMEL MILK

On October 10, 2007, 2 weeks before my son's tenth birthday, he drank his first half cup (4 oz) of thawed raw unheated camel milk. I chose this course because I had spent the previous 2 years studying camel milk and consulting people familiar with its use. In fall 2005, a camel farmer spoke to me about the use of camel milk in Middle Eastern hospitals for premature babies due to its reputed nonallergenic and nutrient-rich qualities. That information led me to theorize the milk might strengthen my son's immune system and thus improve his functioning and also serve as an alternative dairy product. I reviewed the scant literature that evening and over the next few months. In 2006, I found Dr Reuven Yagil's brief 2005 report on several children with ASD responding positively to camel milk. I then consulted Israeli-American scientist Amnon Gonenne, PhD, on his theory that camel milk may act as an anti-inflammatory agent and might help my son. Reassured by anecdotal reports and conversations with healthcare providers and camel milk producers, I concluded the risk of trying camel milk was minimal. One of my son's physicians signed a letter authorizing his need to consume camel milk. I then arranged to receive bottles of raw frozen camel milk from Israel. The camel milk was tested for the presence of bacteria prior to freezing, stored at –20° C, and then shipped by air to me.

On the morning after my son ingested camel milk, he demonstrated astonishing improvements in behavior including eye contact, communication, emotional expression ("I really love you; you're awesome; you do so much for me"), and self-organization. He ate breakfast more neatly, noted his schedule, put on his shoes, and got his backpack for school while conversing at the same time.

He continued consuming 4 oz of camel milk daily with rapid continued improvement in behavior and motor planning. For example, he started looking both ways when crossing streets and parking lots. His erratic behavior stopped, and my frequent offerings of extra protein, which had only somewhat mitigated the problem, were no longer needed. Within 3 weeks, there was also a marked improvement and smoothing of his skin condition. Increasing the daily amount of camel milk to 8 oz seemed to cause new facial grimaces and jerking in one arm, which disappeared when his intake returned to 4 oz. His pragmatic language and vocabulary skills were improved, and other academic skills tested above average and exceptional in some areas.

Interruption of camel milk consumption on several occasions resulted in behavioral and physiological lapses. Just before he turned 12, while I was away from home for two and a half weeks, he did not take camel milk. His school behavior deteriorated to the point that he was in danger of being moved to a special education classroom. Within 24 hours of resuming the camel milk intake, he returned to prior functioning levels. From age 12 to 16 years (present age), he continued on variable amounts of camel milk from Israel and later from the United States, along with conventional medications.

Camel milk has offered observable and sustained benefits to my son's health and functioning. Along with medications and dietary management, I believe camel milk has contributed to the successful management of his symptoms. My son views camel milk positively and is reassured to know he can always access it.

A MOTHER'S PERSPECTIVE

Children with ASD present multiple lifelong challenges. For such a catastrophic and increasingly prevalent disorder, medical treatment and care is debatable, confusing, and expensive. My son's immune and behavioral responses often correlated to dietary matters. Camel milk, a natural food suitable for premature infants, intrigued me as possibly having inherent value as a health and food substance. Camel milk as a trial treatment seemed less invasive and costly than specialist care, medications, alternative treatments, and behavioral interventions.

Just as importantly, camel milk's history gave me assurance. Camel milk has been used for centuries as a medicine in Middle Eastern, Asian, and African cultures. Nomadic cultures have reported living off

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camel milk exclusively with no apparent loss of health. The United Nations lauded camel milk's nutritional content in 2006.[1]

Although anecdotal information on camel milk exists for a variety of illnesses, documented data related to autism are scarce. Jodie Dashore, a board-certified doctor of occupational therapy in private practice in the United States, has begun documenting behavioral outcomes of ASD children with cormorbidities who are ingesting raw camel milk from the United States.

Global attention on the assessment, causes, and treatment of ASD continues to provide parents of autistic children with hope.

My message to parents and physicians would be as follows:

- Intuition of parents and/or patients is critical to pursuing connections between symptoms and potential treatments.
- Communicate all symptoms, even those that seem minute or insignificant, to healthcare providers.
- Affected parents and patients often know when a behavior or symptom is unusual or suspicious.
- Conduct "due diligence" on all therapies, work in partnership with credentialed health providers to assess and ensure safety of new therapies, and always introduce new therapies methodically.
- Document the course of treatment and data from life events with dates and times.
- Camel milk is an available food product with potential therapeutic value. It tastes "just like milk" and can be flavored to preference.

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CAMEL MILK AGAINST AUTISM – A PRELIMINARY REPORT

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ABSTRACT

The described trial substantiated the observation of physicians and parents, that after consuming pasteurised camel milk on a regular basis, a positive effect on impairments of various nature and proportions appeared to be apparent amongst a group of children with Autism Spectrum Disorder (ASD) symptoms or related neurological pathogenesis. Fourteen days after the consumption of 500 ml of pasteurised camel milk, the probands exhibited regular bowel movements and five of eight probands developed a normal sleep pattern. The overall observation revealed also a decreased hyperactivity, increased alertness, better social interaction and many parents observed a newly expressed effort of their children to obey instructions. However, there was no difference in the level of β-casomorphin-7 excretion in the urine of probands and controls using a non-commercial ELISA kit. The reason for this unexpected result is explained.

Key words: Autism, BCM 7, camel milk, casomorphin ELISA

Autism is a collection of behavioral symptoms characterised by dysfunction in social interaction and communication in affected children. It is typically associated with restrictive, repetitive, and stereotypic behavior and manifests within the first 3 years of life. The cause of this disorder is not known (Horvath and Perman, 2002) and should be investigated as a variant of behaviour (Mottron, 2011).

The etiology of Autism is unknown. Several factors have been implicated in its pathogenesis, including genetic, environmental, immunological and neurological elements (Parcell, 2011).

Recent clinical studies have revealed a high prevalence of gastrointestinal symptoms, inflammation, and dysfunction in children with autism. Mild to moderate degrees of inflammation were found in both the upper and lower intestinal tract (Horvath and Perman, 2002)

Panksepp (1979) theorised that components of Autism might be due to excessive opiate activity.

The 'opioid peptide excess' hypothesis described by Dettmer *et al* (2007) postulates, that excessive amounts of endogenous or exogenous opioid peptides, derived from dietary (cow milk) proteins, may be pathophysiologically important in Autism. Casein proteins are incompletely metabolised in the intestine of Autism Spectrum Disorder (ASD) subjects, due to deficient enzyme activity. As a result, short neuroactive peptides – such as B-casomorphins, derived from casein, are formed. B-casomorphin-7 (BCM 7) has long been considered a risk factor for Autism, but the hypothesis remains controversial. Many children with Autism suffer from digestive disorders, which may make them susceptible to BCM 7 absorption (Woodford, 2011).

BCM-7 has also been suggested as a possible cause of sudden infant death syndrome. In addition, neurological disorders, such as autism and schizophrenia, seem to be associated with milk consumption and a higher level of BCM-7. Therefore, careful attention should be paid to that protein polymorphism, and deeper research is needed to verify the range and nature of its interactions with the human gastrointestinal tract and whole organism (Kamiński *et al,* 2007).

Components of camel milk have been described in various publications by different authors, defining the bacteriostatic and virucidal activities as further outstanding attributes, which contributed to the activities of protective proteins like lysozymes, immunoglobulins, lactoferrin and lactoperoxidase

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(Kappeler, 1998). The presence and high amount of these proteins help explain some of the natural healing properties of camel milk.

Gluten or casein elimination diets are beneficial in children with ASDs, which is discussed in numerous publications, books, and parent/lay Autism conferences. Such elimination diets make them less vulnerable to the neuro-active peptides derived from casein/gluten protein.

Camel milk does not contain beta - lactoglobulin found in cow milk, and a different beta-casein (Shabo *et al,* 2005), the two components in cow milk that are responsible for allergies. The whey proteins of camel milk show a different electrophoretic behaviour than those of other species. Differences also exist in the amino acid sequence of camel and cow milk ß-casein, altogether making camel milk beneficial to subjects with various physical and mental disorders. A group of researchers at the Central Veterinary Research Laboratory investigated the medicinal powers of camel milk in a controlled study amongst a group of children, which were believed to suffer from Autism Spectrum Disorders (ASD).

Materials and Methods

Control group and Probands

Fourteen children were selected for this study, of which 6 formed a control group and 8 children were the chosen probands.

The control group consisted of 3 girls and 3 boys. Their age varied between 2 and 7 years and they were personally known to the CVRL researchers and 8 probands group comprised of 2 girls and 6 boys, varying in age between 5 to 21 years. According to the director, all of the 8 probands were known as suffering from symptoms of ASD. But only during the study it became apparent, that two participants (proband G and proband H) could not be clearly described as being ASD sufferers.

The medical details of each proband were as follows:

A - 8 year-old girl

Bowel movement usually only 1 time in 2-3 days, hyperactive, seizures, no social interaction, and difficulties in constructing sentences. There were conflicting statements in regard to the medical condition, which varied between brain abnormality and mild Autism.

B - 13 year-old boy

Bowel movement usually only 1 time in 2-3 days, suffered from serious constipation, extremely hyperactive, little social interaction, speech incoherent, cravings for chocolate. The child was diagnosed with high functioning mild Autism, and was on a casein-free (CF) diet before the start of the trial.

C - 11 year-old boy

Bowel movement normal, hyperactive, does not speak, only gestures, no concentration, can only perform one task at the time, very irritable, frequent outbursts of anger. The child did not meet the usual criteria for Autism. His impairments might be due to a fall of his mother at 8 month in her pregnancy.

D - 5 year-old boy

Bowel movement only one time in 3 days, constipation, irregular sleep pattern, increased hyperactivity, no interaction with people around him unless addressed directly, very irritable, suffered under regular flu-like symptoms, craving for bread. The child was diagnosed with Pervasive Development Disorder (PDD).

E - 11 year-old boy

Attention Deficit Hyperactivity Disorder (ADHD) symptoms prominent, seizures, craving for sweets.

F - 12 year-old boy

Bowel movement one time in 2 days, constipation, irregular sleep pattern, words are repetitive expressed, difficulties in understanding meaning of words. The child was diagnosed with Mild Autistic Syndrome.

G - 14 year-old boy

The child was diagnosed with Hyper Adeno Corticotropic Hormone Deficiency, because of being hyperactive, impaired in social interaction and communication and his repetitive behaviour. He was potassium deficient, his mental growth was slow and he suffers under epileptic seizures.

H - 21 year-old female

Wheel chair bound because of progressive nerve degeneration.

Trial Design

Control group

Urine samples from the 6 control children were collected for a period of 7 days. The samples were immediately frozen at -80° C at CVRL. The urine of the control children were analysed with the aim of establishing the base line values of BCM 7. No special dietry intervention was assigned to this group and all the children were on cow milk products.

Proband group

The duration of the study for the probands was 9 weeks and conducted in 2 phases.

Phase 1 was the 1st week of the trial (week-0). During this period no special dietary intervention was assigned to the probands. During this period, the morning urine was collected daily by their mothers in sterile containers.

Phase-2 was the remaining 8 weeks (week 1 to week 8), during which camel milk was consumed. All 8 probands were provided with 500ml pasteurised camel milk per day, which was consumed without any problems and tolerated well. Parents were notified to refrain from giving their children cow milk products. The collection of morning urine samples of all 8 probands (week 1 – week 8) was conducted every alternative day, including weekends.

All urine samples were transported to CVRL within 2 hours after collection in cool condition. At CVRL, the urine samples were frozen at -80°C until tested.

The effects of camel milk in children with ASD in this trial were measured in two ways.

The excretion of BCM 7 in urine samples was determined using the casomorphin ELISA kit from Immundiagnostik, Bensheim, Germany.

From the start until the end of the experiment after 8 weeks, behavioural and physiological changes of the children participating were daily monitored by their parents and reported weekly to the CVRL researchers.

Two urine samples from each child in the control group and 4 urine samples from each proband in Phase-1 were tested. Six urine samples from each proband in Phase-2 (week 3,6,8) were selected, which comprised of 2 urine samples of different days from week 3, 6 and 8. Samples were analysed for BCM 7 with casomorphin ELISA kit.

The β-casomorphin ELISA test is a competitive enzyme linked immunoassay (cELISA) and the test procedure was carried out according to the manufacturer's recommendation, which is described in brief.

Diluted urine samples and a polyclonal casomorphin antiserum were incubated in microtitre plate wells, coated with a casomorphin derivative (tracer). During the incubation, the target casomorphin of the sample competes with the tracer for the binding of the polyclonal antibodies. The bound components were detected by peroxidase-conjugated antibody and the tetramethylbenzidine (TMB) is used as the secondary reagent. The enzymatic reaction was terminated by an acidic stop solution. The intensity of the yellow colour was inversely proportional to the casomorphin concentration in the sample.

A dose response curve of absorbance unit (optical density, OD at 450nm) vs. concentration was generated using the values obtained from standards. The ELISA results were normalised to the creatinine concentration of the urine sample and the result was expressed in ng/µmol creatinine.

Results

Table 1 shows the BCM 7 values in the control group and Table 2 summarises the BCM 7 values of all 8 probands in Phase 1 (week-0) and Phase 2 (week 3, 6 and 8). The figures in both tables show, that the casomorphin levels in the control and proband groups were within the normal range of 0 - 0,8 ng/µmol creatinine provided by the manufacturer.

The comparitive study of the BCM 7 results in Table 1 and Table 2 indicate that there is no statistical difference between BCM 7 levels of the probands before consuming camel milk and after consumption, as well as between probands and controls.

Although all BCM 7 values were in the normal range of 0 - 0,8 ng/µmol creatinine, we found a significant difference ($P < 0.01$) between the mean values of proband urine samples before and after camel milk consumption. No significant difference (P

Table 1. BCM 7 ELISA results of 6 controls with mean values and standard deviation (SD).

Controls	Sample	BCM 7 concentration (ng/µmol creatinine)
Control 1	Sample 1	0.35
	Sample 2	0.27
Control 2	Sample 1	0.35
	Sample 2	0.26
Control 3	Sample 1	0.31
	Sample 2	0.34
Control 4	Sample 1	0.37
	Sample 2	0.29
Control 5	Sample 1	0.29
	Sample 2	0.50
Control 6	Sample 1	0.24
	Sample 2	0.50
	Mean ± SD	**0.34 ± 0.08**

Table 2. BCM 7 ELISA results of 8 probands before and after camel milk consumption with mean values and standard deviation (SD).

	Week	Sample	BCM 7 concentration (ng/µmol creatinine)							
			Proband 1	Proband 2	Proband 3	Proband 4	Proband 5	Proband 6	Proband 7	Proband 8
Before camel milk consumption	Week 0	Sample 1	0.25	0.33	0.31	0.43	0.12	0.59	0.29	0.20
		Sample 2	0.26	0.34	0.33	0.37	0.26	0.49	0.04	0.10
		Sample 3	0.21	0.23	0.17	0.17	0.29	0.50	0.12	<0
		Sample 4	0.74	0.33	0.26	0.29	0.39	0.67	0.18	0.15
		Mean±SD	**0.37±0.25**	**0.31±0.05**	**0.27±0.07**	**0.31±0.11**	**0.27±0.11**	**0.56±0.08**	**0.16±0.11**	**0.15±0.05**
	Week	**Sample**	**BCM 7 concentration (ng/µmol creatinine)**							
			Proband 1	**Proband 2**	**Proband 3**	**Proband 4**	**Proband 5**	**Proband 6**	**Proband 7**	**Proband 8**
Aftre camel milk consumption	Week 3	Sample 1	0.42	0.29	0.11	0.40	0.19	0.23	0.22	0.05
		Sample 2	0.18	0.07	0.08	0.13	0.23	0.39	0.12	0.09
	Week 6	Sample 1	0.23	0.09	ND	0.18	0.13	0.67	0.17	ND
		Sample 2	0.16	0.29	ND	0.15	0.08	0.40	0.18	ND
	Week 8	Sample 1	0.17	<0 *	ND	0.21	<0	<0	0.05	0.13
		Sample 2	0.20	0.42	ND	0.13	0.21	0.18	0.25	0.15
		Mean±SD	**0.23±0.1**	**0.23±0.15**	**0.09±0.02**	**0.2±0.1**	**0.17±0.06**	**0.37±0.19**	**0.17±0.07**	**0.11±0.04**

* Not detectable ND: Not done

>0.01) was detected in the mean urine BCM 7 values between control group and proband urine before camel milk consumption.

Review of behavioural and physiological changes

From the second week onwards after the introduction of camel milk to the diets of the probands, the parents claimed, that their children exhibited regular bowel movements. This fact represented already a big improvement of the overall health condition of children, who normally show a discomforting prevalence of gastrointestinal problems. Furthermore, they overall revealed a decreased hyperactivity, increased alertness, grasping power and curiosity, better social interaction and many parents commented on the newly expressed effort of their children to listen and obey instructions.

One of the probands was regularly struck by bouts of flu, which resulted in the need of constant medication. During the 8th week trial period that child showed no recurrence of flu symptoms, even though other family members suffered under the disease. Proband developed a mild discomfort, only once which disappeared quickly without causing the need for medication.

Discussion

Over the last years, a significant increase of Autism cases has been observed in children worldwide. Only anecdotal evidence exists that camel milk may have a healing effect on ASD children.

The release of BCM 7 through enzymatic digestion of bovine β-casein is dictated by different amino acids sequences of this protein. The amino acid present in position 67 of the sequence in β-casein appears to be critical for the release of BCM 7. In the A2 variant of β-casein a proline residue occurs at position 67, whereas the A1 and B variants of β-casein have a histidine residue at this position (De Noni, 2008).

BCM-7 may play a role in the aetiology of human diseases (Kamiński *et al,* 2007).

Cass *et al* (2008) studied whether peptides from wheat or milk were leaking from the gut and making their way into the urine of children with autism and did not find any of these small proteins (peptides) in the urine of the boys with autism or Asperger syndrome.

In our study, no difference in the urine BCM 7 levels of both the control and proband groups was detected. This result was unexpected, since it was reported, that children with ASD, whilst drinking cow milk, have increased levels of BCM 7 in their urine. However, no increased BCM 7 levels were found in the urine of our control as well as in our proband group before drinking camel milk. We therefore could not substantiate the result of other researchers, that children with ASD on cow milk diet excrete elevated BCM 7.

Several reasons could be responsible for this discrepancy:

- For the selection of probands, the CVRL researchers depended on the judgement of the director of the centre for children of special needs and some probands included in the trial might not have suffered from classical ASD
- Prior to this trial, the probands were not assessed for the excretion of casomorphin in their urine, because no prior knowledge of biochemical consequences of dietary influence was known
- The ELISA test is yet not commercially available and most probably it's specificity and sensitivity has to be further evaluated for this kind of experiment
- Autism is a complex disease and other neuroactive peptides as well as various undisclosed factors may be responsible for this disorder

Our preliminary trial clearly has shown, that the consumption of camel milk has a positive effect on behavioural and pathophysiological disorders.

However, the level of BCM 7 excretion in the urine of probands and controls using a non-commercial ELISA kit were in the normal range.

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Treatment of digestive problems appears to have positive effects on autistic behavior. These new observations represent only a piece of the unsolved autism "puzzle" and should stimulate more research into the brain-gut connection (Horvath and Perman, 2002).

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Research Article

Camel Milk as a Potential Therapy as an Antioxidant in Autism Spectrum Disorder (ASD)

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Extensive studies have demonstrated that oxidative stress plays a vital role in the pathology of several neurological diseases, including autism spectrum disorder (ASD); those studies proposed that GSH and antioxidant enzymes have a pathophysiological role in autism. Furthermore, camel milk has emerged to have potential therapeutic effects in autism. The aim of the current study was to evaluate the effect of camel milk consumption on oxidative stress biomarkers in autistic children, by measuring the plasma levels of glutathione, superoxide dismutase, and myeloperoxidase before and 2 weeks after camel milk consumption, using the ELISA technique. All measured parameters exhibited significant increase after camel milk consumption ($P < 0.5$). These findings suggest that camel milk could play an important role in decreasing oxidative stress by alteration of antioxidant enzymes and nonenzymatic antioxidant molecules levels, as well as the improvement of autistic behaviour as demonstrated by the improved Childhood Autism Rating Scale (CARS).

1. Introduction

Autism spectrum disorder (ASD) is a severe neurodevelopment disorder with onset prior to 3 years of age [1, 2]. It is characterized by impairments in social orientation, communication, and repetitive behaviors [3, 4]. In addition to behavioural impairment, ASD is associated with high prevalence of autoimmune disease [5, 6], gastrointestinal disease and dysbiosis [7], and mental retardation [8].

The prevalence of autism has increased over the last several decades. The incidence of ASD in United States increased in 2008 to 1 in 88 children [9]. Prevalence of autism spectrum disorders in Saudi Arabia is estimated to be 6 : 1000 [10]. Increased prevalence has great effects on public health implications and has stimulated intense research into potential etiologic factors.

Although the aetiology and pathology is poorly understood, different factors have been suggested to affect autism, for example, immune factors, environmental, neurochemical, and genetic factors [3, 10, 11], oxidative stress [10–13].

Extensive studies have demonstrated that oxidative stress plays a vital role in the pathology of several neurological diseases such as Alzheimer's disease [14], Down syndrome [15], Parkinson's disease [16], schizophrenia [17], bipolar disorder [18], and autism [10, 14].

Oxidative stress occurs when reactive oxygen species (ROS) levels exceed the antioxidant capacity of a cell. It acts as a mediator in brain injury, strokes, and neurodegenerative diseases [19–21]; thus, the control of ROS production is necessary for physiologic cell function. The ROS within the cells are neutralized by antioxidant defence mechanisms, including superoxide dismutase (SOD), catalase, and glutathione peroxidise (GSH-Px) enzymes. The increased production of ROS both centrally (in the brain) and peripherally (in the plasma) may result in the reduction of brain cell number leading to autism pathology and apoptosis [14, 22].

Several studies have suggested the contribution of oxidative stress to the development of autism. These studies demonstrated the alteration of antioxidant enzymes like GSH-Px, MPO, and SOD, lipid peroxidation, antioxidant proteins as ceruloplasmin and transferrin, and detoxifying metabolites like GSH, as well as antioxidant nutrient vitamins and minerals [10, 11, 13, 23–26].

Camel milk has emerged to have potential therapeutic effects in many diseases such as food allergy, diabetes mellitus [27, 28], hepatitis B [29], autism [30], and other autoimmune diseases [31]. It has a unique composition that differs from other ruminants' milk. It contains lower fat, cholesterol, and lactose than cow milk, higher minerals (calcium, iron, magnesium, copper, zinc, and potassium) and vitamins A, B2, E, and C compared to cow milk [32, 33], and it contains no beta lactoglobulin and beta casein which are the main causative of allergy in cow's milk [34]. Furthermore, camel milk contains various protective proteins, mainly enzymes which exert antibacterial, antiviral, and immunological properties [35, 36]; these include immunoglobulins, lysozymes, lactoferrin, lactoperoxidase, N-acetyl-§-glucosaminidase (NAGase), and peptidoglycan recognition protein (PGRP) [34], which are crucial in preventing food allergy and rehabilitating the immune system [31]. Camel milk proved its potential effect in the treatment of food allergies, due to its inflammation-inhibiting proteins, and hypoallergenic properties, in addition to its smaller size nanobodies, which are different than those found in human. Camel milk nanobodies, as a single domain, show many promising and therapeutic potencies in infection and immunity [37].

The aim of the current study was to evaluate the effect of camel milk consumption on oxidative stress biomarkers in autistic children, by measuring the plasma levels of glutathione, superoxide dismutase, and myeloperoxidase.

2. Materials and Methods

2.1. Subjects. The present study included 60 subjects with ASD, especially those with known allergies or food intolerances, aged 2–12 years. Clinical diagnosis was based on the criteria for autistic disorder as defined in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV) [2]. Subjects were recruited from the Autism Research and Treatment Center, Faculty of Medicine, King Saud University.

The study protocol received the ethical approval from the Institutional Review Board of Faculty of Medicine, King Saud University. A written informed consent was obtained from all parents/guardians before being enrolled in the study.

2.2. Study Design. The study was a double-blinded, randomized clinical trial (RCT). The participants were randomly divided into three groups: Group I ($n = 24$) received raw camel milk; Group II ($n = 25$) received boiled camel milk; and Group III ($n = 11$) received cow milk as a placebo. All groups received the same instructions, volume of milk, and containers to preserve the blinding of the study.

Parents were instructed to include the average of 500 mL of camel milk in their children's regular daily diet for a period of 2 weeks. Parents were asked to continue with the children's daily routines. They were not allowed to add or remove any interventions such as diet plans, supplements, or pharmacotherapies throughout the study period. Group I was also instructed to drink cold milk, beginning with small quantities that increase gradually, until 500 mL per day was consumed to avoid any risk of diarrhoea.

2.3. Childhood Autism Rating Scale (CARS). The Childhood Autism Rating Scale (CARS) was administered as a measure of symptom severity [11]. The Wing Subgroups Questionnaire (WSQ) [12] is a questionnaire with 13 behavioral domains (e.g., communication, social approach, play, imitation, motor behavior, and resistance to change) on which parents rate their child's behavior. A summary score is calculated for each subtype (i.e., aloof, passive, and active but odd), and the highest summary score is considered to indicate the subtype.

2.4. Blood Sampling. After overnight fast, ten mL blood samples were collected in EDTA tubes from autistic children before and 2 weeks after camel milk consumption. Centrifugation was done; plasma and red blood cells were obtained and deep frozen (at −80°C) until further analysis.

2.5. Methods

2.5.1. Measurement of Glutathione. This was done by using commercially available ELISA kit (Wuhan Eiaab Science Inc., China) specific for measurement of plasma glutathione levels according to the manufacturer's instructions. Briefly, the microtiter plate has been precoated with an antibody specific to GSH. Standards and samples were pipetted into the wells with a biotin-conjugated polyclonal antibody specific for GSH. Next, avidin conjugated to horseradish peroxidase (HRP) was added and incubated. A substrate solution was added and colour developed in proportion to the amount of GSH. The colour development was stopped, and the intensity of the colour was measured.

2.5.2. Measurement of Superoxide Dismutase. This assay employs the quantitative sandwich enzyme immunoassay technique for the assessment of human superoxide dismutase in plasma (Wuhan Eiaab Science Inc., China). A monoclonal antibody specific for SOD has been precoated onto a microplate. Standards and samples were pipetted into the wells, followed by addition of a second antibody specific for SOD. Then, a substrate solution was added to the wells and colour developed in proportion to the amount of SOD bound in the initial step. The colour development was stopped, and the intensity of the color was measured.

2.5.3. Measurement of Myeloperoxidase. Plasma myeloperoxidase level was measured using double antibody sandwich ELISA (GenWay biotech, USA) according to the manufacturer's instructions. This method is based upon formation of enzyme-labeled antibodies complex followed by addition of

Table 1: Glutathione, superoxide dismutase, and myeloperoxidase in plasma of autistic children together with CARS before and 2 weeks after camel milk consumption.

	Raw milk ($N = 24$)		Boiled milk ($N = 25$)		Placebo ($N = 11$)	
	Mean ± SEM	P value	Mean ± SEM	P value	Mean ± SEM	P value
Glutathione						
Before	0.37 ± 0.03	0.05	0.34 ± 0.03	0.02	0.36 ± 0.02	0.5
After	0.41 ± 0.01		0.45 ± 0.02		0.35 ± 0.04	
SOD						
Before	0.54 ± 0.03	0.2	0.49 ± 0.02	0.007	0.52 ± 0.03	0.5
After	0.59 ± 0.02		0.57 ± 0.02		0.54 ± 0.03	
MPO						
Before	2.65 ± 0.17	0.05	2.44 ± 0.13	0.02	2.11 ± 0.37	0.2
After	3.22 ± 0.24		3.08 ± 0.19		2.62 ± 0.16	
CARS						
Before	37.63 ± 6.31	0.004	36.82 ± 3.27	0.001	34.18 ± 3.25	0.772
After	34.54 ± 5.19		33.80 ± 4.91		34.41 ± 3.25	

chromogenic substrate to develop a color that is proportionate to the myeloperoxidase concentration.

2.6. Statistical Analysis. The data were analyzed and presented as mean ± SEM (standard error of the mean). Statistical differences in each measurement before and 2 weeks after milk therapy were determined with P values, and $P < 0.5$ was considered significant. The receiver operating characteristics (ROC) curve as a fundamental tool for biomarkers evaluation was performed using the same computer program. In a ROC curve, the true positive rate (sensitivity) is plotted in function of the false positive rate (100-specificity) for different cut-off points of a parameter. Each point on the ROC curve represents a sensitivity/specificity pair corresponding to a particular decision threshold. The area under the ROC curve is a measure of how well a parameter can distinguish between camel-milk-treated and untreated autistic subjects.

3. Results

The present study was performed to study the effect of camel milk consumption on oxidative stress on ASD subjects by measuring the plasma levels of glutathione, superoxide dismutase, and myeloperoxidase.

Table 1 and Figure 1 show plasma levels of GSH, SOD, and MPO together with CARS of autistic children, before and 2 weeks after camel milk consumption. All measured parameters showed significant changes after camel milk consumption.

Plasma GSH levels were significantly increased in group I and group II ($P = 0.05$, $P = 0.02$, resp.), but not in group III, following 2 weeks of camel milk consumption. In addition, plasma levels of SOD demonstrated no significant differences in group I ($P = 0.2$) and group III ($P = 0.5$). On the other hand, group II demonstrated a highly statistically significant change following 2 weeks of boiled camel milk consumption ($P = 0.007$). Furthermore, there was a significant elevation of MPO in both group I, the raw camel milk ($P = 0.05$), and group II, the boiled camel milk ($P = 0.02$), but not in group III, the placebo group ($P = 0.2$).

Table 2 and Figures 2(a)–2(d) demonstrate ROC analysis of the 4 measured variants. It could be easily noticed that GSH, SOD, MPO, and CARS show higher area under the curve (AUC), % specificity, and sensitivity in groups I and II than in group III.

4. Discussion

The present study aimed at evaluating the effect of camel milk on oxidative stress among subjects with autism spectrum disorders by measuring the levels of antioxidant enzymes: SOD, MPO, and GSH.

Several studies have suggested an increased vulnerability of subjects with ASD to oxidative stress. Oxidative stress and the consequent damage occur when antioxidant defence mechanisms fail to effectively counter endogenous or exogenous sources of reactive oxygen species [38]. Increased oxidative stress might contribute to behavioural aberrations, sleep disorder, and gastrointestinal disturbances in autistic children [39, 40].

Low plasma antioxidant enzymes, GSH-Px [25] and SOD [23], were reported. Low level of antioxidant enzymes indicated increased vulnerability to oxidative stress due to impaired antioxidant defence mechanisms, which lead to harmful effects of free radicals that could have an important role in the aetiology of autism. Moreover, increased oxidative stress in autistic subjects leads to a decrease in the levels of nonenzymatic antioxidants like GSH, vitamin E and C [13], which in turn leads to impairment of metabolic pathways and may contribute to the developmental delays which occur in autism; this could be corrected by micronutrient supplementation [41]. In addition, lower plasma levels of glutathione and cysteine in subjects with ASD were documented [42, 43].

Camel milk has been reported to improve clinical outcomes of ASD [31]. The effect of camel milk consumption on autistic behaviour was documented through significant changes in the Childhood Autism Rating Scale (CARS) scoring results [44], as casein- and gluten-free diet has been reported to improve autistic behavior [31], possibly by reducing excess central opioid effects [45].

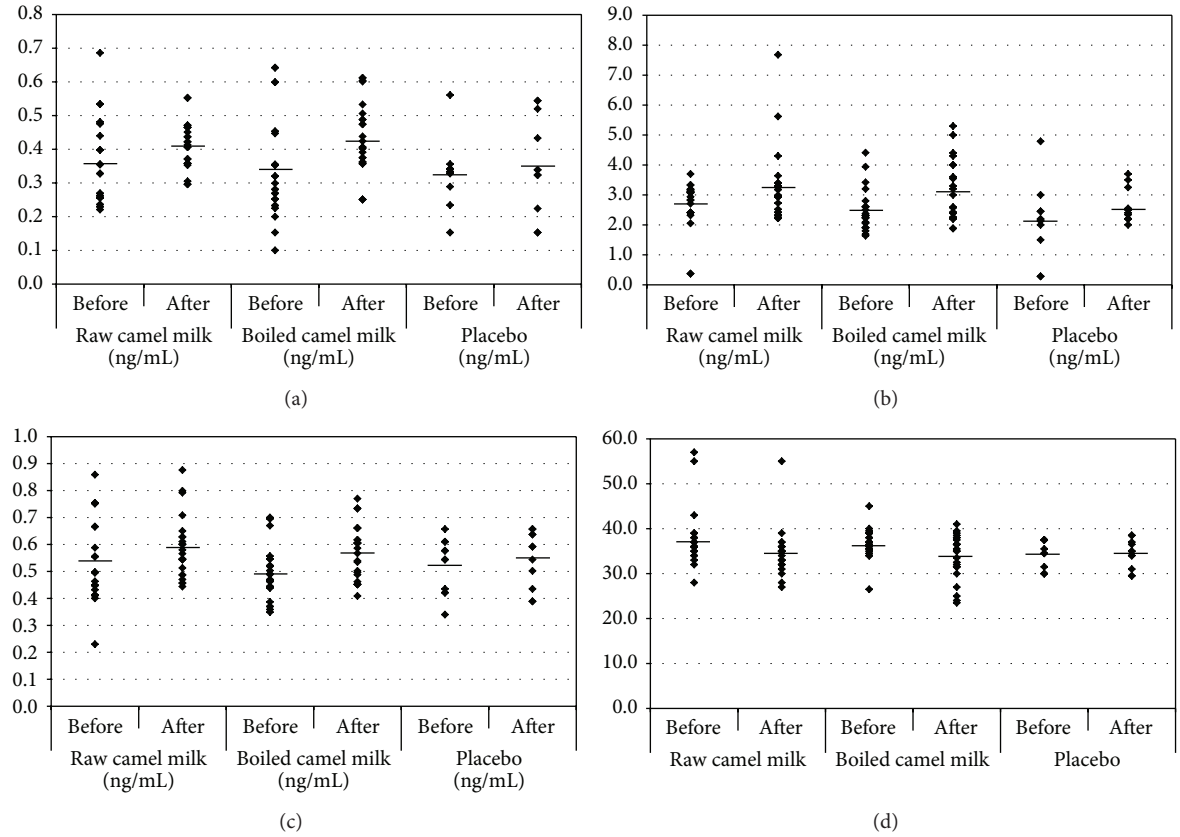

Figure 1: Levels of (a) GSH, (b) SOD, (c) MPO, and (d) CARS in autistic patients before and after treating with the camel milk. The mean value for each group is designated by a line.

Table 2: ROC curve of GSH, SOD, MPO, and CARS of autistic patients before and after treatment with camel milk.

Parameters		Raw camel milk	Boiled Camel milk	Placebo
GSH	Area under the curve	0.677	0.723	0.504
	Best cut-off value	0.357	0.356	0.326
	Sensitivity%	83.3	88.0	45.5
	Specificity%	62.5	72.0	72.7
SOD	Area under the curve	0.642	0.706	0.591
	Best cut-off value	0.453	0.562	0.585
	Sensitivity%	95.8%	56.0%	54.5
	Specificity%	41.7%	88.0%	72.7
MPO	Area under the curve	0.584	0.703	0.702
	Best cut-off value	3.17	2.385	2.180
	Sensitivity%	45.8%	76.0%	90.9%
	Specificity%	79.2%	64.0%	54.4%
CARS	Area under the curve	0.729	0.682	0.512
	Best cut-off value	35.5	33.75	37.25
	Sensitivity%	70.8	44.0	81.8
	Specificity%	70.8	96.0	36.4

Glutathione is one of the most important intracellular antioxidants, responsible for maintaining the reducing intracellular microenvironment that is essential for normal cellular function and viability. It also exerts neuroprotective properties and reduces neuropathy and hence decreases oxidative stress.

Subjects with ASD were shown to exhibit abnormal plasma levels of metabolites in the pathway of glutathione redox metabolism, due to inefficient detoxification system [12]. The concentration of reduced glutathione (GSH) was found to be significantly decreased compared to control [10, 25], which reflects increased oxidative

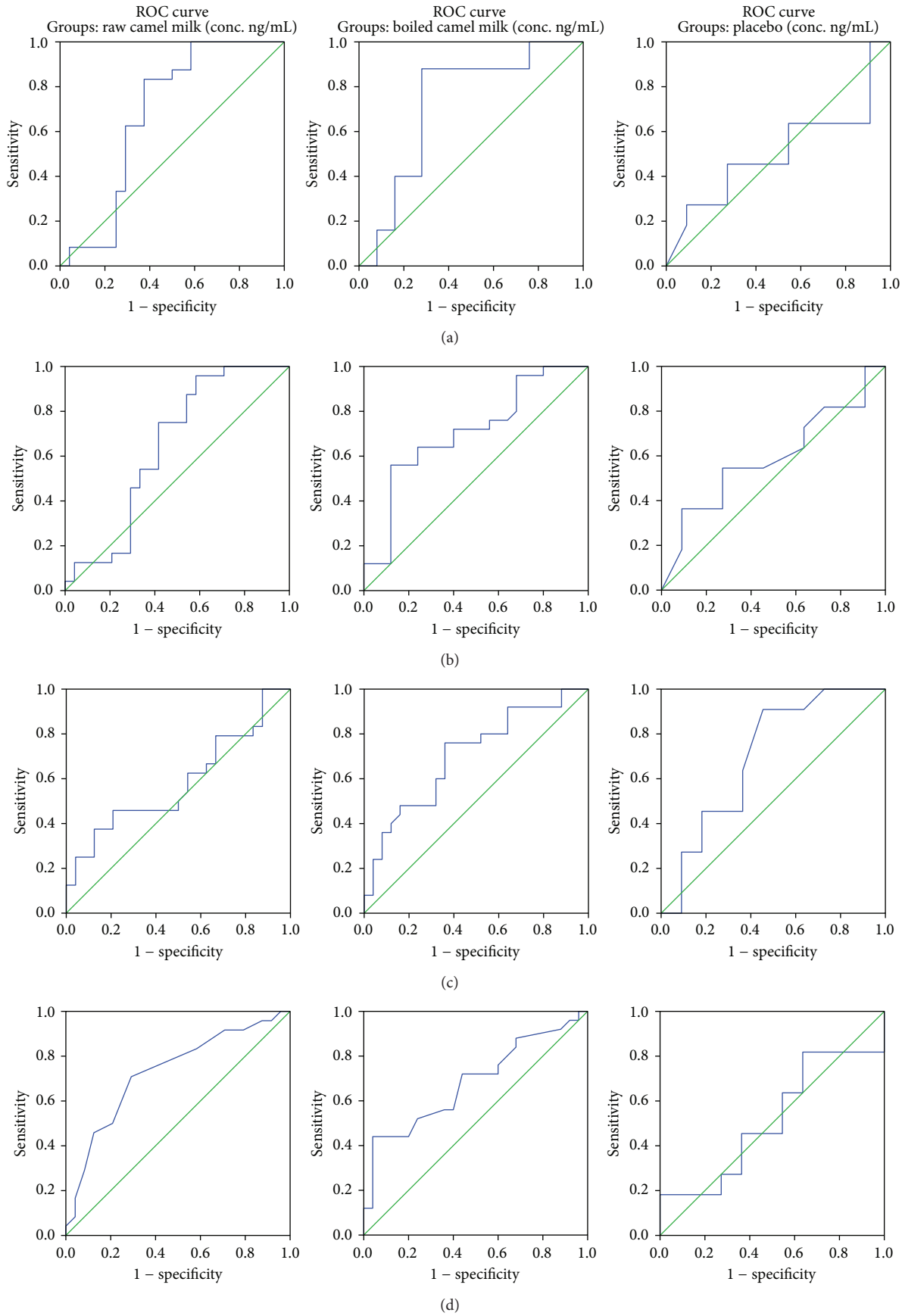

Figure 2: ((a)–(d)): ROC curves showing specificity, sensitivity, and area under the curves for (a) GSH, (b) SOD, (c) MPO, and (d) CARS.

stress due to the impaired defense mechanisms against ROS.

The results of the present study show a significant increase in GSH level after camel milk consumption; this could be attributed to the antioxidant nutrients constituents of camel milk. Magnesium is known to reduce oxidative stress and enhance vitamin E and C absorption [44], whereas zinc increases total glutathione, GSHPx, and SOD levels. Moreover, vitamin E has been suggested to enhance glutathione levels [46]. Taken together, high levels of Mg and Zn and vitamin E in camel milk might help to increase glutathione production and enzymes production and hence to decrease the oxidative stress in autistic subjects.

Superoxide dismutase is an antioxidant enzyme that inhibits lipid peroxidation by catalyzing the conversion of superoxide into hydrogen peroxide (H_2O_2) and oxygen (O_2) [13] and acts as a primary defence, as it prevents further generation of free radicals. Insufficient capacity of SOD to metabolize the resulting H_2O_2 may lead to toxicity [10].

It was shown that the SOD activity was significantly higher in autistic children compared to control, in response to oxidative stress. The increased activity may be an adaptive response to eliminate superoxide that was excessively produced [10]. In contrast, other studies reported significant decrease of SOD levels in autistic children compared to controls [24, 43], due to the impairment of the defence mechanism against oxidative stress. Low SOD may also contribute to the nutritional status as some of the antioxidant nutrient levels affect the status of the antioxidant enzymes. For example, adequate amounts of superoxide dismutase are produced when the body receives an adequate and balanced intake of copper and zinc. Copper deficiency was reported to reduce the level of superoxide dismutase [23, 46], whereas zinc deficient diet decreases superoxide dismutase, glutathione peroxidase, total glutathione, and vitamin E [47]. Other studies suggested that the low zinc levels have been associated with autism and related to lower SOD levels, due to the lower zinc to copper ratio in autistic children compared to controls [23, 48].

In the present study, SOD level was significantly increased after camel milk consumption; this could be attributed to the high contents of zinc, copper, magnesium, and vitamin E in camel milk.

Myeloperoxidase is a biomarker of oxidative stress that is responsible for microbicidal activity against a wide range of organisms and one of the indicators of inflammation [49]. Elevated superoxide generated from dysfunctional mitochondria promotes the formation of excessive H_2O_2, the substrate for MPO-mediated hypochlorous acid synthesis, which is then converted to the inflammatory biomarker, 3-chlorotyrosine (3-CT), in activated immune cells during an inflammatory response [38].

Elevated expression of MPO has previously been demonstrated in chronic neurological disease states, such as Alzheimer's disease [50], Parkinson's disease [51], multiple sclerosis [52], and autism spectrum disorder [53].

It has been demonstrated that autistic children with severe GI disease have low serum levels of MPO, which is directly linked with GI pathology seen in this group [54]. The present study demonstrated a significant increase in the plasma myeloperoxidase level following camel milk consumption, which could be a consequence of increased level of SOD. MPO and SOD work synergistically to protect the cell contents against oxidizing activity by destroying anions and hydrogen peroxide [50]; superoxide dismutase catalyzes the conversion of superoxide radicals to H_2O_2, with catalase neutralizing H_2O_2 and then myeloperoxidase converting H_2O_2 to highly reactive hypochlorous acid [23]. Another possibility might be the improvement of GI problems due to the deprivation of camel milk from beta lactoglobulin and beta casein, the major cause for food allergy and GI disease in autistic subjects [7, 54, 55].

Various studies demonstrated a remarkable improvement of some symptoms in ASD subjects following a gluten- and casein- free diet [34], glutathione supplementation [22], antioxidant supplementation such as vitamin E, C, and selinum [22–24], or magnesium and zinc supplementation [43]. These molecules are essential for glutathione synthesis, antioxidant enzymes activities, antioxidant vitamins absorption, and effective antioxidant defence mechanism and hence they play an important role in decreasing oxidative stress as confirmed in various studies.

In light of this information, the role of camel milk in decreasing oxidative stress and treatment of ASD could be explained on the basis that it contains high level of antioxidant vitamins C, A, and E and is very rich in antioxidant minerals magnesium and zinc. Antioxidant vitamins are useful in reducing the oxidative stress. Vitamin E and magnesium have been suggested to enhance glutathione biosynthesis. Magnesium deficiency has been associated with the production of reactive oxygen species [46]. On the other hand, zinc is essential for the activity of many enzymes in living organisms such as SOD and GPx. It has been reported that zinc can prevent cell damage through activation of the antioxidant system [47, 56]. Taken together, these nutrients enhance the production of detoxifying molecules, absorption of antioxidant vitamins, and activation of antioxidant enzymes which in turn activate the detoxification system and reduce the exerted oxidative stress. Another possibility is that camel milk can help to combat and treat gastrointestinal problems, which are frequently associated with ASD, due to its inflammation-inhibiting constituents and hypoallergenic properties, in addition to its smaller size antibodies which are similar to human antibodies [7, 37], and thus improve some autistic behaviours.

The role of the measured parameters in the etiology of autistic features could be also ascertained in this study. The amelioration induced by raw and camel milk on GSH, SOD, and MPO was accompanied by a significant improvement in the behaviour of the autistic children after two weeks of camel milk consumption. CARS was significantly lower after camel milk consumption than before.

Table 2 and Figures 2(a)–2(d) demonstrate that although the four measured parameters did not show very high specificity and sensitivity, GSH and CARS show satisfactory values of both measures. This could help to suggest GSH as a predictive biomarker to follow the potency of camel milk

treatment in parallel with the measurement of CARS as a behavioural and cognitional measure.

In conclusion, our findings suggest that camel milk could play an important role in decreasing oxidative stress by alteration of antioxidant enzymes and nonenzymatic antioxidant molecules levels and improvement of autistic behaviour. A larger scale study considering the period and dosage of camel milk is needed to determine the effect of camel milk on oxidative stress biomarkers and hence the treatment of ASD. In addition, other parameters representing different signalling pathways related to the pathology of autism are recommended. Screening for a predictive marker which might record higher specificity and sensitivity than those of the present study is critically needed.

Conflict of Interests

The authors have no conflict of interests to disclose.

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