

Evaluation Of The Efficacy And Safety Of Probiotic Formulation With Zinc Enriched Yeast In Children With Acute Diarrhea

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Citation

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Abstract

Background: Diarrhea is a common and potentially serious illness in early childhood. A child suffers, on an average, 10 to 15 episodes of diarrhea in the first five years of life. Particularly in developing countries like India this problem is much more common and also severe due to poor hygiene and less accessible healthcare facilities compared to western world. **Objective:** The objective of the study was to evaluate the efficacy and safety of a probiotic formulation with Zinc enriched yeast in children with acute diarrhea. **Materials and Methods:** This was an open label, non-randomized clinical trial conducted on outpatient basis by the qualified investigators at five different centers. A total of 104 pediatric patients (6-60 months of age) with acute diarrhea of < 48 hrs duration were enrolled in the study. The enrollment of the patients was as per inclusion/exclusion criteria. After obtaining informed consent they were individually examined, investigated and treated as per study protocol. The treatment included daily one sachet of Lactobacillus rhamnosus Rosell-11 (1Billion cells), Saccharomyces boulardii (125mg) along with Zinc enriched yeast (equivalent to elemental Zinc 20mg) for 10- 14 days.

Results: Within studied population, 60% patients with acute diarrhea showed complete recovery from their symptoms by day 3, 35% by day 5 and remaining 5% of patients by day 10. These patients showed symptomatic improvement in all studied parameters which included, both stool frequency and consistency, episodes of vomiting and urination frequency. It was observed that 95 % of patients resumed normal food intake & activities in 5 days. As per investigator's assessment, the efficacy of the treatment with trial medication was good and very good in 89 patients and satisfactory in 11 patients. Among the 100 patients studied, 20 patients rated the therapy with the trial medication as very good, 69 patients as good and 11 patients as satisfactory. All the patients reported good tolerability of the formulation with no serious adverse events. **Conclusion:** The present study concludes that once a day administration of probiotic-zinc combination containing Lactobacillus rhamnosus Rosell-11, Saccharomyces boulardii and zinc enriched yeast is effective in the treatment of acute diarrhea and associated symptoms.

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INTRODUCTION

Acute diarrhea is a sudden change in stool fluid output that increases the number of bowel movements to more than three per day. Diarrhea contributes significantly to infant morbidity and mortality, with the World Health Organization (WHO) estimating that 3 million deaths occur per year as a result of diarrhea.^[1] In developing countries, children younger than 5 years of age have an average of 3 episodes of diarrhea per year, compared with 1 to 2 episodes per year in the United States.^[2] Almost one in every five children in India below the age of 14 suffers from diarrhea.

Acute diarrhea is most commonly caused by infections. Bacterial infections are more apparent in the early months of infancy, whereas from 6 months to 2 years of age, rotavirus accounts for 25% to 40% of acute diarrheal cases^[3]. There are many other potential causes of acute diarrhea including drug therapy, food allergies, gastrointestinal disorders, and malnutrition.

Diarrhea is a common but potentially serious illness in early childhood. A child may lose almost as much water and electrolytes from the body during an episode of diarrhea as an adult, since the length and surface area of intestinal mucosa of a child, from where the diarrhea fluids are secreted, are fairly large. Loss of one liter of fluid from the

body of a child weighing 7 Kg is much more hazardous compared with a similar depletion from an adult of 70 Kg weight. Significant dehydration disturbing the electrolyte balance and acid-base status of the body occurs in about 2 to 3 per cent of all cases of diarrhea. Some of these cases may prove fatal, if fluids and electrolytes are not replaced to restore normal circulatory levels and body functions which are impaired in the dehydrated state.^[1]

Probiotics have been studied for the management of various types of diarrheal disease, including viral diarrhea, bacterial diarrhea, antibiotic-associated diarrhea, and *Clostridium difficile* diarrhea. The use of the term probiotic for living organisms dates back to 1989, when they were regarded as a supplement of living microorganisms that bring a health benefit by improving the balance of the intestinal microbiota. Probiotics are living organisms, which when administered in adequate amount; confer a health benefit on the host.

Probiotics are non-pathogenic microorganisms and are considered to be a safe and effective part of the first-line therapy for acute diarrhea in children and adults.^[4] Probiotic microorganisms positively change the intestinal flora, inhibit the growth of pathogenic bacteria, promote adequate digestion, stimulate local immune function and increase resistance to infection. Probiotics exert their beneficial effects by a variety of complementary mechanisms, including an ability to modulate host systemic & local immune responses, secretion of mucin from intestinal goblet cells and competitive exclusion of pathogens.^[4] In addition, previous studies have shown that probiotics have the ability to decrease *Citrobacter rodentium* - induced mucosal inflammation and disruption of colonic paracellular junctions.^[4]

Lactobacillus is a gram-positive, lactic acid-producing bacterium that was first isolated from the stools of a healthy human.^[5] Various strains of *Lactobacillus* have been evaluated for their health benefits. *L. rhamnosus* has been employed experimentally as a supplement to the human neonatal intestinal microflora and the result reveals its effects on the enhancement of immunoglobulin (Ig) secretion. This probiotic prevents rotavirus-induced diarrhea, and the recurrence of colitis, and it protects against Indomethacin-induced changes in barrier function both in humans and in polarized intestinal cell monolayers grown in tissue culture.^[5] A notable characteristic of this bacterium is its ability to adhere to epithelial cells in tissue culture and

displace intestinal pathogens, including *E. coli*.^[5]

L. rhamnosus Rosell-11 is a strain of *Lactobacillus* with improved stability and efficacy profile. In vitro tests have shown that *L. rhamnosus* Rosell-11 is able to inhibit the adhesion of pathogens to intestinal epithelial cells & promote their eradication. This *L. rhamnosus* strain has shown effectiveness in the treatment of both diarrhea and chronic constipation (Tlaskal 1995). *L. rhamnosus* Rosell-11 grows on lactose, thus helps to reduce lactose intolerance in children.

Saccharomyces boulardii belongs to the group of simple eukaryotic cells (such as fungi and algae) and, it thus differs from bacterial probiotics that are prokaryotes.^[32] *S. boulardii* favorably alters the composition of the gut flora and inhibits the action of pathogenic microorganisms. It maintains a healthy balance of intestinal flora by producing organic compounds such as lactic acid, hydrogen peroxide and acetic acid that increase the acidity of the intestine and inhibit the reproduction of many harmful bacteria. Studies have revealed that it also produces substances called bacteriocins, which act as natural antibiotics to kill undesirable microorganisms. It has a well demonstrated role in the synthesis of vitamin B1, vitamin B2, vitamin B6, pantothenic acid and nicotinic acid. These vitamins are utilized for the nutritional requirements of the body.^[32]

Zinc supplementation: - Since zinc and probiotics work via different mechanisms it is possible that adding both would have a synergistic effect. In addition to probiotics, intervention trials have demonstrated that the addition of oral zinc can also reduce the duration and severity of acute diarrhea in children.^[7,11,12] The rationale for the beneficial effect of zinc supplementation is based on the depletion of zinc due to diarrhea^[11, 13] and the deleterious effects of zinc deficiency on the immune system, leading to more severe enteric infections.^[14] The possible mechanisms for the effect of zinc supplementation on diarrhea include improved absorption of water and electrolytes by the intestines,^[9,10,11] regeneration of gut epithelium or the restoration of its function,^[16,17,18,19] increased levels of enterocytic brush-border enzymes,^[22,24,25] and enhanced immunologic mechanisms for the clearance of infection, including cellular immunity and higher levels of secretory antibodies.^[30,31,32]

In 2004 WHO and UNICEF released a joint statement for treatment of acute diarrhea recommending therapeutic zinc (20 mg/day) for 10–14 days along with oral rehydration salts

containing lower concentrations of glucose and salt.

Different forms of zinc have been evaluated for the efficacy. Zinc-enriched yeast is special yeast which can provide ample organic zinc. Zinc-enriched yeast is derived from cultures of specified strains of *Saccharomyces cerevisiae* grown in the presence of zinc chloride or zinc sulphate. Fermentation takes place at a specified temperature and pressure for defined periods of time. This is followed by increasing the temperature to kill the yeast. The cell wall is ruptured to release the contents which are then spray dried to form zinc enriched yeast. In addition to zinc, it also contains micronutrients like vitamins and other minerals. Organic form of zinc is more bioavailable compared to other available zinc formulations. One of the studies demonstrated that zinc from zinc-enriched yeast was 3.7 times more bioavailable, i. e. absorbed and found in greater concentration in the liver than the zinc gluconates. ^[35]

The objective of the present study was to evaluate the efficacy and safety of probiotic formulation with zinc enriched yeast in children with acute diarrhea.

MATERIALS AND METHODS

This was an open label, non-randomized clinical trial to assess the efficacy and safety of probiotics with zinc formulation in the treatment of acute diarrhea in children. The test formulation was supplied by Aristo Pharmaceuticals Pvt. Ltd., Mumbai. Each dose of test medication contains *Lactobacillus rhamnosus* Rosell-11: 1 billion cells, *Saccharomyces boulardii*: 125mg and zinc enriched yeast: equivalent to elemental zinc 20mg.

The study was conducted in 104 pediatric patients with acute diarrhea at five different sites.

After independent ethics committee approval, eligible patients were administered with test formulation and frequency and consistency of stools were recorded daily for the duration of treatment up to 10 days.

METHODOLOGY

Children of age 6-60 months with acute diarrhea (< 48 hrs) reporting for the consultation to the investigators were selected for the study. After thorough physical and clinical examinations, subjects who fulfilled inclusion / exclusion criteria and parents/guardian willing to sign the informed consent form were enrolled into the trial. The inclusion criteria included written consent from parents of the patients,

patients who will comply with the procedures and requirements of the study, parents of patients able to read, write, follow instructions and record the data in parent's daily diary. Patients were excluded from the study in case of severe dehydration requiring hospitalization and I.V. fluids, coexisting acute systemic illnesses, food allergy or other chronic gastrointestinal diseases, use of probiotics in the previous three weeks and diarrhea with blood visible in stools.

Trial medication was administered once a day and efficacy parameters and adverse effects if any were recorded. As per the WHO guidance ORS supplementation was given to study population at an average of 7 unit doses/24 hrs. Patients were monitored by the parents and reported to the physician on 3rd, 5th and 10th day. Patient's diary was filled by the parent /legal representative. Measured efficacy parameters included, Stool frequency, Consistency of stool, Frequency of urination in 24 hours, Episodes of vomiting and ORS consumption.

Treatment was continued for 10 days even after the patients were completely cured to complete the zinc supplementation course as per WHO guidelines.

STATISTICAL ANALYSIS

The data was then compiled, and subjected for statistical analysis. The data was analyzed as average (mean) during treatment period. For efficacy parameters mean score of baseline was compared with mean score of day 3, day 5 and day 10. T-test (two tails) was applied to compare the efficacy of the treatment on day 0, day 3, day 5 and day 10. For nonparametric symptoms like consistency of stools ANOVA test was applied.

RESULTS

The total of 104 patients were enrolled in the study, 100 patients completed the study and were considered for final analysis.

A) Demographic profile: Out of 100 children, 40 were females and 60 were males with average age among male patients was 18 months and among the female patients was 20 months. Majority of the patients were in the weight group of 6 -10 kg. Among the patients enrolled in the trial, minimum weight was 6.7 kg and maximum was 19 kg. At the base line visit, out of 100 patients with diarrhea, 9 patients had fever, 12 patients had vomiting and 29 patients had fever along with vomiting.

B) Efficacy parameters: Complete recovery with acute diarrhea was observed in 60% of patients by day 3, 35% by day 5 and remaining 5% of patients by day 10.

1. Day 0 – day 3 (n=60): Out of 100 patients, 60% of patients with acute diarrhea recovered from symptoms by day 3. The frequency of stools decreased from mean score 7.5 on day 0 to 2.5 on day 3. On day 0 the stools were watery which returned to normal consistency by day 3. Frequency of urination increased from mean 4.68 to 6.86 on day 3. Mean score for vomiting was 3.41 on days 0 and completely stopped by day 3.

Figure 1

Table: 1- Efficacy parameters of patients recovered completely at the end of day 3

	PARAMETERS	Day 0	Day 3	p-value
A	No. of stools in 24 hrs.	7.5	2.5	3.95E-22
B	*Consistency of stools	3.00	1.00	-
C	Frequency of urination in 24 hrs.	4.68	6.86	2.10E-07
D	Episodes of vomiting	3.41	0	2.87E-05
[*Normal (well formed=1, Semisolid=2, Watery=3)]				

2. Day 0 - day 5 (n=35): By day 5, 35% of the patients were completely cured of the symptoms. Mean score of frequency of stools was 8.31 on day 0 which reduced with the test medication to 2.66 on day 5. Mean score of consistency of stools was decreased from 3.00 (watery) on day 0 to 2.1 (Semi solid) by day 3 and stools get normalized by the day 5 with a mean score of 1.00. Frequency of urination increased from mean 4.89 on day 0 to 8.74 on day 5. Mean score for vomiting was 2.39 on day 0 and completely stopped by day 5.

Figure 2

Table: 2- Efficacy parameters of patients recovered completely at the end of day 5.

	Parameters	Day 0	Day 3	Day 5	p-value
A	No. of stools in 24 hrs.	8.31	5.2	2.66	4.51E-15
B	*Consistency of stools	3.00	2.10	1.00	
C	Frequency of urination in 24hrs.	4.89	6.54	8.74	2.1E-07
D	Episodes of vomiting	2.39	1.33	0	1.2E-05
E	ORS consumption (sachets/day)	5.29	4.43	1.80	2.67E-08
[*Normal (well formed=1, Semisolid=2, Watery=3)]					

3. Day 0 - day 10 (N=05): The remaining 5% of patients with acute diarrhea were symptom-free by day 10. Frequency of stools reduced from 11.6 on day 0 to 1.8 by day 10. On day 0, stools were watery which gradually changed to semisolid by day 3 and had normal consistency by day 10. Frequency of urination was also significantly increased from day 0 (6.20) to day 5 (8.40) and then slightly declined to 8.25 by day 10. Episodes of vomiting which occurred initially were cured by day 5 completely. ORS consumption was also found to be reduced from 7.6 sachets in 24 hours to 1.00 on day 10.

Figure 3

Table: 3- Efficacy parameters of patients recovered completely at the end of day 10.

	Parameters	Day 0	Day 3	Day 5	Day 10	p-value
A	No. of stools in 24 hrs.	11.6	5.2	2.6	1.8	0.092557
B	*Consistency of stools	3	2.25	2	1	----
C	Frequency of urination in 24 hrs.	6.20	7.80	8.40	8.25	0.294256
D	Episodes of vomiting	1.67	1.00	0	0	0.089009
E	ORS consumption (sachets/day)	7.60	5.40	4.00	1.00	0.0717604
[*Normal (well formed=1, Semisolid=2, Watery=3)]						

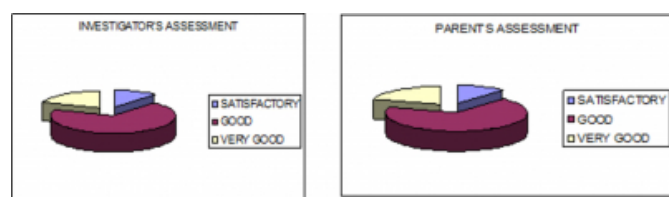
C) Safety evaluation: Of 100 patients studied, no adverse events were observed in children. Initially 36 patients had fever which was cured by the second and third day of treatment, 12 patients had vomiting along with diarrhea which was cured by day 5. None of the patients opted to withdraw from the study due to occurrence of any adverse event.

D) Investigator's assessment of therapy: Most of the investigators rated the therapy as above satisfactory. Up to 90% investigators think that the probiotic enriched zinc is a good or very good choice for the treatment of diarrhea.

E) Parents assessment of therapy: Among the parents of 100 patients studied, 20% rated the therapy with the trial medication as very good, 69% as good and 11% parents as satisfactory.

Figure 4

Figure: 4- Investigator's and Parent's assessment of therapy



DISCUSSION

The aim of this multicentric, prospective, phase III clinical trial was to investigate the therapeutic efficacy and safety of orally administered probiotic formulation with zinc enriched yeast in treating acute diarrhea in children. The results showed that probiotic formulation with zinc enriched yeast showed improvement in terms of both time to response and response rate.

Acute diarrhea in children is very often self-limiting within few days. However, children are in danger of developing dehydration and a deteriorating general health. Therefore, an effective antidiarrheal treatment would be beneficial. Several investigations have been carried out with probiotics for the treatment of acute gastroenteritis, and different meta-analyses and systematic reviews have been published in this field. All of these have demonstrated the efficacy of probiotics in treating or preventing diarrhea. On an average, the treatment of diarrhea with lactobacilli, bifidobacteria and/or *S. boulardii* shortened the duration of diarrhea by 0.5–1.5 days.^[32, 33]

Szajewska and Mrukowicz reviewed ten randomized, double-blind, placebo-controlled studies and concluded that the administration of probiotics led to a substantial reduction in the duration of acute diarrheal symptoms, an average of 20 h.^[33] Moreover, a meta-analysis of nine clinical trials conducted by D'Souza et al. demonstrated that probiotics effectively prevented antibiotic-associated diarrhea.^[32] The work of van Niel et al. included nine randomized controlled studies with lactobacilli in acute infectious diarrhea in children. In these studies, the duration of diarrhea was significantly reduced by an average of 0.7 days along with the reduced daily stool frequency.^[34] More recently, McFarland et al. examined the efficacy of probiotics in pediatric diarrhea by analyzing 39 randomized, controlled and blinded clinical trials comprising a total of 41 probiotic treatment arms.^[31] Of these, 32 (78%) reported efficacy. The latest meta-analysis of 39 trials by Szawal et al. showed that probiotics prevented acute diarrhea, with a risk

reduction among children of 57% (range: 35–71%).^[32]

In the present trial 104 patients were enrolled, 100 patients completed the trial successfully. Patients who did not come for follow up visit (4 patients) were considered as drop outs. Primary outcome analyzed in this study is duration of diarrhea, frequency and consistency of stools.

All the patients had watery stools and had an average of 8 episodes of diarrhea in 24 hours. Out of 100 patients, 66 patients had moderate dehydration and 4 had severe dehydration. Out of 66 moderately dehydrated patients, 42 (63%) patients were cured by day 3, 22 patients (34%) by day 5 and 2 patients (3 %) by day 10. The 4 severely dehydrated patients were cured by day 5. The rest 5% were cured by day 10 because all the 5 patients were underweight and had malnutrition.

On an average, 60% of patients were cured by day 3 and 35 % were cured by day 5 and the remaining 5 % by day 10. Frequency of stools decreased to almost normal in 24 hrs.

Among 100 patients with diarrhea, 12 patients had vomiting (average 3 episodes in 24 hours), 9 patients had fever and 29 patients had vomiting and fever. Frequency of urination was 4 on an average in 24 hours at base line. On an average, seven unit doses of ORS were consumed by patients in 24 hrs on the day of enrollment. In the present study, decrease in frequency of vomiting was observed which may not be due to medication but overall reduction in disease process and ORS administration. The test medication was well tolerated by the patients with no serious adverse events reported.

CONCLUSIONS

This clinical trial clearly demonstrates that the probiotic-zinc combination containing *Lactobacillus rhamnosus* Rosell-11, *Saccharomyces boulardii* and zinc enriched yeast provided clinical benefits to the patients of acute diarrhea and showed improvement in terms of both the time to response and response rate to diarrhea. The primary outcome was a statistically significant improvement in frequency and consistency of stools. The secondary outcomes were reduction in other symptoms like vomiting, fever and other signs of dehydration. Probiotic usage resulted in early recovery and prevented prolongation of diarrhea and secondary malabsorption. No adverse events were observed with the usage of the studied antidiarrheal medication. Larger population studies are needed as confirmatory trials

for *L. rhamnosus* Rosell-11 as well as zinc enriched yeast in the antidiarrheal treatment.

References

- Guandalini S. Acute Diarrhea. Pediatric Gastrointestinal Disease, 3rd Edition, Eds. Walker WA, Hamilton JR, Watkins JB, Durie PR, Walker-Smith JA 2000, BC Decker.
- World Health Organization, The World Health Report 1995.
- Glass RI, Lew JF, Gangarosa RE, LeBaron CW, Ho MS. Estimates of morbidity and mortality rates for diarrheal diseases in American children. *J Pediatr* 1991; 118(4 (Pt 2): S27-33.
- Ghishan FK. Transport of electrolytes, water, and glucose in zinc deficiency. *J Pediatr Gastroenterol Nutr* 1984;3:608-612.
- Patrick J, Golden BE, Golden MHN. Leucocyte sodium transport and dietary zinc in protein energy malnutrition. *Am J Clin Nutr* 1980;33:617-620.
- Understanding and Managing Acute Diarrhoea in Infants and Young Children (health education to villages) Health and Family Welfare India New Delhi - 110- 011
- Atrick J, Michael J, Golden MN, Golden BE, Hilton PJ. Effect of zinc on leucocyte sodium transport in vivo. *Clin Sci Mol Med* 1978;54:585-587.
- Golden BE, Golden MHN. Zinc, sodium and potassium losses in the diarrheas of malnutrition and zinc deficiency. In: Mills CF, Bremner I, Chesters JK, eds. Trace elements in man and animals — TEMA 5. Aberdeen, United Kingdom: Rowett Research Institute, 1985:228-32.
- Golden BE, Golden MHN. Dietary zinc and the output and composition of faeces. In: Gawthorne JM, Howell JM, White CL, eds. Trace element metabolism in man and animals. Berlin, Germany: Springer-Verlag, 1982:73-6.
- Roy SK, Draser BS, Tomkins AM. The impact of zinc deficiency on the intestinal response to cholera toxin. *Proc Nutr Soc* 1986;45:39A-39A.
- Bettger WJ, O'Dell BL. A critical physiological role of zinc in the structure and function of biomembranes. *Life Sci* 1981;28:1425-1438.
- Elmes ME, Jones JG. Ultrastructural changes in the small intestine of zinc deficient rats. *J Pathol* 1980;130:37-43.
- Arcasoy A, Akar N, Ors U, Delilbasi L, Karayalcin S. Ultrastructural changes in the mucosa of the small intestine in patients with geophagia (Prasad's syndrome). *J Pediatr Gastroenterol Nutr* 1990;11:279-282.
- Koo SI, Turk DE. Effect of zinc deficiency on the ultrastructure of the pancreatic acinar cell and intestinal epithelium in the rat. *J Nutr* 1977;107:896-908.
- Moran JR, Lewis JC. The effects of severe zinc deficiency on intestinal permeability: an ultrastructural study. *Pediatr Res* 1985;19:968-973.
- Ford RPK, Menzies IS, Phillips AD, Walker-Smith JA, Turner MW. Intestinal sugar permeability: relationship to diarrheal disease and small bowel morphology. *J Pediatr Gastroenterol Nutr* 1985;4:568-574.
- Weaver LT, Chapman PD, Madeley CR, Laker MF, Nelson R. Intestinal permeability changes and excretion of micro-organisms in stools of infants with diarrhoea and vomiting. *Arch Dis Child* 1985;60:326-332.
- Evans PH, Bates CJ, Lunn PG, et al. zinc supplementation in young Gambian children. *Proc Nutr Soc* 1993;52:25A-25A.
- Roy SK, Behrens RH, Haider R, et al. Impact of zinc supplementation on intestinal permeability in Bangladeshi children with acute diarrhoea and persistent diarrhoea syndrome. *J Pediatr Gastroenterol Nutr* 1992;15:289-296.
- Gebhard RL, Karouani R, Prigge WF, McClain CJ. Effect of severe zinc deficiency on activity of intestinal disaccharidases and 3-hydroxy-3-methylglutaryl coenzyme A reductase in the rat. *J Nutr* 1983;113:855-859.
- Jones PE, Peters TJ. Oral zinc supplements in non-responsive coeliac syndrome: effect on jejunal morphology, enterocyte production, and brush border disaccharidase activities. *Gut* 1981;22:194-198.
- Suzuki I, Kushida H. Studies on mammalian glycosidases. V. Effects of metal ions upon acidic and neutral -glucosidases, -galactosidases, and -mannosidases in liver extracts of rabbit. *J Biochem (Tokyo)* 1973;74:627-629.
- Peters TJ. Analytical subcellular fractionation of jejunal biopsy specimens: methodology and characterisation of the organelles in normal tissue. *Clin Sci Mol Med* 1976;51:557-574.
- Lisowski J, Rajkumar TV, Wolf DP, Stein EA. Evidence for tightly-bound zinc in leucine aminopeptidase from pig kidney. *Acta Biochim Pol* 1970;17:311-324.
- Warren L, Glick MC, Nass MK. Membranes of animal cells. I. Methods of isolation of the surface membranes. *J Cell Physiol* 1966;68:269-288.
- Truding R, Shelanski ML, Daniels MP, Morell P. Comparison of surface membranes isolated from cultured murine neuroblastoma cells in the differentiated or undifferentiated state. *J Biol Chem* 1974;249:3973-3982.
- Cunningham-Rundles C, Cunningham-Rundles S, Garofalo J. Increased T lymphocyte function and thymopoietin following zinc repletion in man. *Fed Proc* 1979;38:1222-1222.
- Fenwick PK, Aggett PJ, Macdonald D, Huber C, Wakelin D. zinc deficiency and zinc depletion: effect on the response of rats to infection with *Trichinella spiralis*. *Am J Clin Nutr* 1990; 52:166-172.
- Schlesinger L, Arevalo M, Arredondo S, Diaz M, Lönnerdal B, Stekel A. Effect of a zinc-fortified formula on immunocompetence and growth of malnourished infants. *Am J Clin Nutr* 1992;56:491-498.
- D'Souza AL, Rajkumar C, Cooke J, Bulpitt CJ. Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis. *BMJ* 2002;324:1361-1366.
- McFarland LV, Elmer GW, McFarland M. Meta-analysis of probiotics for the prevention and treatment of acute pediatric diarrhea. *Int J Probiotics Prebiotics* 2006;1:63-76.
- Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE. Efficacy of probiotics in prevention of acute diarrhea: a meta-analysis of masked, randomized, placebo-controlled trials. *Lancet Infect Dis* 2006;6:374-382.
- Szajewska H, Mrukowicz JZ. Probiotics in the treatment and prevention of acute infectious diarrhea in infants and children: a systematic review of published randomized, double-blind, placebo-controlled trials. *J Pediatr Gastroenterol Nutr* 2001;33:S17-S25.
- Van Niel CW, Feudtner C, Garrison MM, Christakis DA. Lactobacillus therapy for acute infectious diarrhea in children: a meta-analysis. *Pediatrics* 2002;109:678-684.
- Thomas A, Tompkins, Nadine E. Renard and Akira Kiuchi Clinical Evaluation of the Bioavailability of Zinc-enriched Yeast and Zinc Gluconate in Healthy Volunteers. *Biological Trace Element Research* 2007; 120 (1-3), 28-35

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