IMPACT OF ZINC FORTIFIED YOGHURT ON ACUTE PNEUMONIA IN CHILDREN

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ABSTRACT

Zinc (Zn) has a role in the acute phase response to infection helping to boost the body's immune response through a defense cascade. The objective was a trial to assess the effect of Zn supplemented yoghurt, when given with routine hospital treatment, on reducing treatment failure, length of hospital stay, and duration of symptoms of pneumonia in children less than five years old. 45 children suffered from acute severe pneumonia were subjected to complete physical exam and randomized to receive either Zn supplemented yoghurt 20 mg Zn-acetate "= 6 mg Zn element" (group-B, n=15) or 20 mg Zn element "= 66 mg Zn-acetate" (group-C, n=15) per 240 ml yoghurt divided into 2 or 4 meals/day according to child appetite) or placebo (group-A, n=15) and followed up until they were discharged from the hospital. Serum Zn, hemoglobin percent and C-Reactive Protein were measured once on admission (before supplementation) for all and serum Zn on discharge (after supplementation) for group-B and group-C only.

The results revealed that there was no statistical significant difference between the 3 manufactured zinc-supplemented yoghurt samples as regard chemical and physical properties and no statistical significant difference between the 3 groups as regards age, sex, length/height, weight, nutritional history, clinical picture and serum Zn on admission. There was no mortality in the 3 groups. Serum Zn was significantly increased in group-B & group-C on discharge while hospital days for improvement and total hospital stay were shorter in group-B and group-C than placebo. Hospital day for improvement in group-A is significantly longer than that of group-B and highly

significantly than in group-C while the total hospital days in group-A is highly significantly longer than that of group-C only. There was no significant negative correlation between serum Zn after and hospital days for improvement and a significant one with total hospital days.

***Conclusively**, from these results it could be concluded that Zn-supplemented yoghurt decreases the duration of hospital stay, and duration of symptoms of severe pneumonia in best treatment dose of 6mg/day and more effectively in dose of 20 mg/day of Zn as nutrient element.*

Keywords: Zinc Fortified Yoghurt, Acute Pneumonia, Children.

INTRODUCTION

Zinc is an essential element, necessary for sustaining all life. It is estimated that 3000 of the hundreds of thousands of proteins in the human body contain zinc prosthetic groups. Zinc is found in oysters, and to a far lesser degree in most animal proteins, beans, nuts, almonds, whole grains, pumpkin seeds and sunflower seeds. Phytates, which are found in whole grain breads, cereals, legumes and other products, have been known to decrease zinc absorption. Zinc deficiency results from inadequate intake of zinc, or inadequate absorption of zinc into the body (Aydemir *et al.,* 2006).

Experience with lower mortality and morbidity due to infectious disease in well-designed, randomized, controlled trials of zinc supplements in young children has highlighted zinc deficiency as a public health problem of global proportions (Jones *et al.,* 2003). Zinc administered as a therapeutic agent to young children with acute or persistent diarrhea also reduces the duration of the diarrhea and is associated with a lower rate of treatment failure or death (Bhutta *et al.,* 2000).

However, results of studies of zinc administered as adjuvant therapy for pneumonia have been more limited.

Zinc might act in the acute phase response to infection (Ling *et al.,* 1996 and Gaetke *et al.,* 1997) helping to boost the body's immune response through a defence cascade, beginning with mobilisation and sequestration of zinc to metallothionein-rich tissue, rapid up regulation of immune defence-specific protein synthesis, activation of immune defence activity such as macrophages, lymphocytes, and natural killer cells, and antibody-dependent cytotoxicity (Cousins, 1985).

Aim of study:

It was to assess the effect of zinc supplemented yoghurt, when given with antibiotics, in duration of symptoms of severe pneumonia, length of hospital stay and change of serum zinc level in children less than five years old.

Subjects:

This is a double blind randomized placebo-controlled clinical study done in the Pediatric Zagazig University, In patient Hospital from Sep. 2008 to Sep. 2010. Hundred children suffered from severe pneumonia between 2 and 5 years old at the time of admission were randomized to receive zinc supplemented yoghurt or plain yoghurt plus the hospitals standard antimicrobial management until discharge.

The age-group 2–5 years was selected for its ability to feed , high pneumonia morbidity and restricted options to prevent community-acquired bacterial pneumonia. A fixed dose of 20 mg of elemental zinc per day was chosen because this is the dose being adopted for programmatic use in diarrhea case management (Fontaine, 2001). Additionally the median mg/kg exposure for this age-group was estimated to fall well below the range of acute toxicity (Fosmire, 1990).

A study physician did a baseline physical examination, including timing of respiratory rate, assessment of breathing effort, cyanosis, mental status, and chest auscultation of crepitation or wheezing, or both. Axillary temperature was taken with a mercury thermometer.

Inclusion criteria: Children aged 2–5 years were considered eligible for enrollment in the study if they fulfilled the criteria for a diagnosis of severe bacterial pneumonia. Severe pneumonia was defined as a respiratory rate more than 40/min, which was accompanied with crepitations on auscultation and the presence of one or more of the following danger signs; lethargy, inability to feed, chest indrawing, or central cyanosis (Bose *et al.,* 2006). Bacterial pneumonia was defined as pneumonia with C-reactive protein concentrations more than 40 mg/L (Coles *et al.,* 2007). Consent was obtained by a study physician who read the entire consent form to the parent.

Exclusion criteria: Children were excluded if they had concurrent diarrhea, were receiving zinc supplements, or had severe malnutrition, inability to feed, history of chronic cardiac or renal disease, illness severe enough to require ventilation and illness requiring hospitalization in the previous 21 days or failure of taking regular 20mg zinc supplemented meals /day.

Treatment groups:

Children were then randomized to three groups: First group-A had been received placebo yoghurt, Second group-B had been received zinc supplemented yoghurt in a dose of 20 mg zinc-acetate that equal 6 mg zinc element per 240 ml yoghurt and third group-C had been received zinc supplemented yoghurt in a dose of 66 mg zinc-acetate that equal 20 mg zinc element per 240 ml yoghurt.

The yoghurt was divided into 2 or 4 meals / day according to child appetite until they were discharged from the hospital. The supplement preparations were administered at the time of enrolment, within 1 h of the first dose of parenteral antibiotics on admission day. The placebo and zinc-supplemented yoghurt were approximately similar in appearance, color, odor, and taste. The zinc and placebo yoghurt were prepared and labeled A, B and C respectively by the researcher.

Follow up:

Data for respiratory rate, chest indrawing, auscultation findings, fever, feeding, cyanosis, and mental status, were obtained at the beginning of every nursing shift (8h). All enrolled patients were managed according to the Hospital's standard severe pneumonia treatment guidelines. Parenteral antibiotics were ampicillin (200–400 mg/kg of body weight per day, given intravenously every 6h) and gentamicin (6–7.5 mg/kg per day, given intravenously every 8h). Patients who failed to improve after 48 h of antibiotics (by the eighth dose of ampicillin) or whose condition worsened, had their antibiotic changed to ceftriaxone (50 mg/kg per day intravenously).

Failure to improve and worsening condition were established by respiratory rate count and severe pneumonia signs. Worsening of any one sign qualified as worsening condition and no change in any sign constituted failure to improve. Although fever was not necessary for diagnosis, it was taken as a sign of active infection, and thus had to be absent for 24 consecutive hours without the aid of antipyretics to qualify for change from severe to non-severe pneumonia.

Severe pneumonia was reclassified to pneumonia (non-severe) when chest indrawing and hypoxia (oxygen saturation less than 95% on room air) were absent for 24 consecutive hours and respiratory rate was less than or equal to 40 breaths per min, at which time oral antibiotics were started. The oral antibiotic was amoxicillin 40 mg/kg divided every 8 h. If any sign recurred, the child was reclassified as severe status until these conditions were met.

Children were discharged from hospital once respiratory rate fell to less than or equal to 40 per min for 24 consecutive hours, with no recurrence of respiratory distress, other danger signs, or fever (temperature $>37.9^{o}$C). Before discharge 3 mL of blood was drown from every child for measurement of post-intervention serum zinc concentration in group B and C.

MATERIALS:

Buffalo's milk:
Fresh whole buffalo's milk was obtained from Dairy Laboratory, Faculty of Agriculture, Zagazig University.

Zinc acetate:
Zinc acetate (extra pure dihydrate) was obtained from Loba Chemie PVT.LTD .www.lobachemie.com(INDIA).

Human blood samples:
Three mL blood sample was obtained from every patient at the time of admission for measuring serum zinc level. Another two ml blood sample was collected in EDTA (ethylenediaminetetraacetic acid) tubes for measuring blood hemoglobin percent.

METHODS:

Bio-yoghurt manufacture:
Yoghurt starter culture (Yo-Flex, YC-350, direct vat starter (DVS)) consists of *Streptococcus thermophilus*, *L. delbrueckii subsp.bulgaricus*; probiotic bacteria, L. acidophilus *La*-5 and *Bifidobacterium (B.) lactis Bb-12 (DVS)* were obtained from Chr. Hansen's Lab A/S Copenhagen Denmark.

Fresh buffalo milk was heated at 90 C for 5 min, then cooled to 37C; inoculated with yoghurt starter, *L. acidophilus La*-5 and *B. lactis Bb*-12 at 0.01, 0.025 and 0.025%, respectively (Chandand and OʻRell, 2006).

The milk was divided into three portions: the first portion (A) was regarded as placebo (plain yoghurt), the second portion (B) was fortified with zinc acetate at a level of 20 mg / 240 ml and the third portion (C) was fortified with zinc acetate at a level of 66mg/240ml. Each portion was distributed in 60ml plastic cups(labeled A orb accordingly) ,the cups closed and incubated at 37 ᵒC until a firm curd was formed (5.5h) then kept in a refrigerator to be served to the patient within 24h.

Chemical analysis and serum zinc:

Zn and major elements concentration in the three yoghurt samples (A, B and C) were carried out in Central Laboratory of Faculty of Veterinary Medicine, Zagazig University using Atomic Absorption Spectrophotometer (model 210VGP, buck Scientific USA) with an oxidizing air acetylene flame (A.O.A.C, 1990). Titratable acidity, total solids, protein and fat were determined according to Ling (1963). pH value was measured using a pH meter (PTI-15, Aqua Chemical Co., England).

Microbiological analysis was done by using selenite cystine broth For Salmonella agar media for total viable count of bacteria, lactic acid bacteria, *Streptococcus; Salmonella; Staphylococcus aureus* and spores incubated both aerobically and anaerobically; MRS agar containing 10mg ml-1 nystatin selective for lactic acid bacteria; M-17 agar selective for Streptococcus, Brilliant green, xylose lysine decarboxylate, and bismuth sulphite agar selective for Salmonella spp.; Mannitol salt agar- selective for staphylococci; Baird Parker agar selective for *Staphylococcus aureus* (Harrigan & Mc Cane, 1976).

Measurement of blood hemoglobin **(Hb%):**

It was performed at Central Laboratory of Faculty of Medicine, Zagazig University by using automated blood cell counter (Sysmex, Sysmex corporation, Kobe, Japan) to all patients within 24h admission.

Statistical analysis:

The statistical calculations were done using Computer Programs Microsoft Excel Version 14.0 (Microsoft Corporation 2010, NY, USA) and SPSS Version 17.0 (Statistical Package for the Social Science; SPSS inc, Chicago, IL, USA) for Microsoft Windows (Andy, 2005).

RESULTS AND DISCUSSION

Data in Table 1 revealed no apparent differences between the three samples as regard to the chemical and physical properties. In agreement with the present results of plain-yoghurt sample were Omer (2004) and Osman (2004) pH, titratable acidity, total solids protein fat, total ash ,Zn, P, Mg and Ca, as well as, flavour, odour, color, and consistency of plain and zinc fortified bio-yoghurt Samples were determined.

Table 1: Chemical composition and physical properties of yoghurt

Yoghurt \ Properties	Plain (A)	Zn acetate Supplemented	
		20mg/240ml (B)	66mg/240ml (C)
Chemical:			
pH	4.39	4.35	4.40
Titratable acidity, gm/100mL	1.13	1.13	1.14
Total solid /100mL	15.30	15.31	15.33
Fat gm/100mL	5.38	5.40	5.39
Protein, gm/100mL	4.70	4.75	4.73
Total ash, gm/100mL	0.80	0.82	0.93
Zn, mg/100mL	0.49	2.9	8.7
P, gm/100mL	9.10	9.09	9.11
Mg, gm/100mL	1.30	1.33	1.31
Ca, gm/100mL	13.70	13.69	13.71
Physical (sensory): Flavor	Normal	Normal	Undetectable metallic
Odour	Normal	Normal	Normal
Consistency	Soft	Soft	Slightly firmer
Color	Whitish	Whitish	Whitish

Values are means of three independent determinations.

On the other hand, Fuentea *et al*. (2003) examined the content of major minerals (calcium, magnesium, phosphorus and zinc) of 16 commercial yoghurts from five different branches and recorded different results than ours due to addition of different dairy products or fractions during manufacturing.

Data in Table 2 revealed no apparent microbiological differences between the three samples of yoghurt because we used only fresh samples (< 48 hr) during this study. Approximately this agreed with Laye *et al*. (2006) who found that viable bacteria concentrations ranged from 140 to 8,000 $\times$ 10^6/g and ratios of lactobacillus to streptococcus ranged from 0.18   to 15.4.

Table 2: Total microbial viable count (cfu g^{-1}) of the three buffalo milk yogurt samples (A, B & C).

Yoghurt / Type of bacteria	Plain (A)	Zn acetate Supplemented	
		20mg/240ml (B)	66mg/240ml (C)
Total bacterial count	2.59×10^5	2.60×10^5	2.59×10^5
Staphylococcus aureus	ND	ND	ND
Streptococcus	1.60×10^6	1.61×10^6	1.61×10^6
Lactobacillus	1.72×10^5	1.73×10^5	1.71×10^5
Coliform	149	150	149
Escherichia coli	62	62	61
Salmonella spp.	ND	ND	ND

Values are means of three independent determinations.
Cfu g^{-1}: Colony-forming units per gram.   **ND:** Not detect.

In Eissa *et al*. (2010) results revealed that the increment of the acidity of the growth media with the storage time may retard the bacterial growth. These results are in agreement with the findings of Masud *et al*. (1991).

Data in Table (3) shows that there is no significant difference between the three groups as regard age, hieght and weight (P > 0.05) by ANOVA test. Also there is no significant difference between the 3 groups as regard sex distribution, nutritional history and pneumonia types by Chi- Square. So we can say that the three groups were homogeneous to exclude the effect of these factors on the course of the disease, as Chia-Ni Lina *et al.* (2012) found that there was no significant effect of gender on serum zinc, which concerned in the present study.

From these results in Table 4, it could be concluded that the three studied groups are homogeneous as regard the severity of the condition (temperature, pulse and respiratory rate) and hemoglobin level by ANOVA test and the clinical manifestations (wheezy chest and signs of dehydration) showed no significant statistical difference by Chi Square test.

So from the previous results in Tables 3 & 4, the baseline characteristics of the children were well balanced between the placebo (group-A) and the zinc treated groups (group-B & group-C).

Table 3: Comparison between the three groups as regard of age, height & weight by ANOVA test and gender, nutritional history & type of pneumonia by Chi- Square test.

Groups / Items	Group A n=15	Group B n=15	Group C n=15	Test	P
Age / mo				**F** 0.31	0.735
Mean ± SD	32.2 ±8.92	32.13 ±6.46	30.30±6.42		
Range	24 - 55	24- 45	24- 45		
Weight / kg				**F** 0.174	0.841
Mean ± SD	12.76 ±1.57	12.83±1.39	12.53±1.40		
Range	11.0 - 16.0	11.0 - 15.5	11.0 - 15.5		
Length / cm				**F** 0.257	0.775
Mean ± SD	90.00±6.95	89.86±6.40	88.66±6.34		
Range	82.0 -103.0	80.0 - 89.0	80.0 - 99.0		
Gender:				χ^2 0.000	1.000
Female	n = 7 (46.7%)	n = 7 (46.7%)	n = 7 (46.7%)		
Male	n = 8 (53.3%)	n = 8 (53.3%)	n = 8 (53.3%)		
Nutr. History:				χ^2 0.189	0.910
Not accepted	n = 5 (33.3%)	n = 6 (40.0%)	n = 6 (40.0%)		
Accepted	n = 10 (66.7%)	n = 9 (60.0%)	n = 9 (60.0%)		
Pneumonia types				χ^2 1.514	0.469
Lober -	n = 7 (47.7%)	n = 6 (40.0%)	n = 6 (40.0%)		
Broncho -	n = 8 (53.3%)	n = 9 (60.0%)	n = 9 (60.0%)		

n: Number, **F:** ANOVA test, **P:** Probability, **SD:** Standard Deviation, **mo:** Month, **g:** Kilogram, **cm:** Centimeter, χ^2 **:** Chi- Square.

Results in Table (5) shows that the serum zinc before the manipulation and duration of disease before admission have no statistical significant difference (P =0.800) between the three groups so the three groups are homogeneous as regard this factor. While the serum zinc after the manipulation (at day of discharge) shows a statistically significant increase in group-C than group-B (P=0.000).

There is a significant difference as regard hospital days for improvement from severity (P = 0.026) by ANOVA. By LSD hospital days for improvement in group-A is significantly longer than that of group-B and group-C while there is no significant difference between group-B and group-C. Also the total hospital admission days shows a significant difference between the three groups by ANOVA test (P = 0.006). By LSD total hospital

Table 5: Comparison among the three groups (Mean ± SD) serum zinc level on day of admission and day of discharge.

Items \ Groups	Group A n=15	Group B n=15	Group C n=15	F-test	P
Serum zinc before (μmol/L)					
Mean ± SD	9.09 ± 0.75	8.87 ± 1.05	8.96 ± 0.86	0.22	0.800
Range	7.9 - 10.3	7.0 – 10.2	7.7 – 10.2		
Serum zinc after (μmol/L)					
Mean ± SD	---	9.60 ± 0.96	11.96 ± 1.02	20.35	0.000*
Range		8.0 – 11.9	10.3 – 13.3		
Duration before admission					
Mean ± SD	2.0 ± 0.92	1.86 ± 0.91	1.86 ± 0.91	0.105	0.900
Range	1 – 4	1 – 4	1 – 4		
Hospital days for improvement					
Mean ± SD	5.66 ± 3.03	4.66 ± 1.11	3.66 ± 0.89	3.987	0.026*
Range	4 - 15	3 – 6	3 – 6		
Total hospital days					
Mean ± SD	7.53 ± 3.62	5.80 ± 1.20	4.73 ± 0.96	5.796	0.006*
Range	5 – 18	4 – 7	4 – 7		

* Significant difference.

n: Number, **F:** ANOVA test, **P:** Probability, **SD:** Standard Deviation, **μmol/L:** Micromole per liter.

Table 6: Paired T test for serum zinc level before and after zinc supplemented yoghurt intake.

Items \ Groups	Group B & C n=30	Group B n=15	Group C n=15
Serum zinc before (μmol/L)			
Mean ± SD	8.920 ± 0.950	8.873 ± 1.058	8.966 ± 0.864
Serum zinc after (μmol/L)			
Mean ± SD	10.786 ± 1.547	9.606 ± 0.966	11.966 ± 1.022
P for paired T- test	0.000*	0.005*	0.000*

* Significant difference.

N: Number, **F:** ANOVA test, **P:** Probability, **SD:** Standard Deviation, **μmol/L:** Micromole per liter.

In general the serum zinc levels in the previous studies of Brooks *et al* (2004), Mahalanabis *et al*. (2004), Bose *et al*. (2006) and Coles *et al*. (2007)

were different than ours because the time of blood collection (which occurred at any hour of the day or night throughout the studies) is known to cause variation in plasma zinc concentrations (Hotz *et al*., 2003).

      In agreement with the present results Coles *et al*. (2007) found that at discharge, mean ($\pm$SD) plasma zinc concentrations were significantly higher in the zinc group than in the placebo group (13.0$\pm$2.5 and 12.0$\pm$4.1 μmol/L, respectively; P= 0.013).

      Correlation test shows that there are none significant negative correlation (P > 0.05) between the zinc level and hospital days of improvement and a significant one (P = 0.006) as regard total hospital days (Table 7).

Table 7: Correlation between serum zinc levels (at day of discharge) of the treated patients with supplemented yoghurt and both of the total days of improvement and the total hospital days

Correlation test	R	P
Hospital days for improvement	-0.336	0.07
Total hospital days	-0.491	0.006*

   * Significant difference.   **P:** Probability   **R:** Correlation

      These results are in line with Brooks *et al*. (2004) who found that children given zinc recovered from severe pneumonia an average of one day earlier than did those given placebo, and their average stay in hospital was one day longer. The zinc supplement was safe and well tolerated. Since a course of zinc treatment costs only US$0.15, and one day staying in hospital costs US$25, the potential cost savings are substantial.

      Conclusively, from these results it could be concluded that the low serum zinc level due to low dietary zinc intake of children in our community suggests that zinc supplements for the treatment of pneumonia are likely to be effective.

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دراسة تأثير الزبادي المدعم بالزنك على صحة الأطفال المصابين بالالتهاب الرئوي الحاد

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تتأثر صحتنا بما نأكله، و يعتبر الزنك من المكملات الغذائيه الأساسيه لإرتباطه بكثير من وظائف الجسم و خصوصا العمليات المتعلقه بالجهاز المناعى، حيث يتواجد الزنك فى تركيب كل الخلايا تقريبا فيحفز نشاط أكثر من مائة انزيم مسئولين عن العديد من التفاعلات الكيميائيه. استهدف هذا البحث اختبار مستوى الزنك فى مصل حالات الإلتهاب الرئوى البكتيرى الشديد بالاطفال و تأثير الإمداد بالأغذيه المدعمه على مستوى الزنك و علاقته بسرعة التحسن و الشفاء. و قد أجرى هذا البحث السريرى المشتمل على عينة مقارنة بمستشفى الأطفال الجامعى بالزقازيق فى الفترة من سبتمبر ٢٠٠٨ حتى سبتمبر ٢٠١٠ و شمل ٤٥ طفلا تراوحت أعمارهم ما بين ٢ ـ ٥ سنه مصابين بالإلتهاب الرئوى البكتيرى الشديد تم تقسيمهم عشوائيا الى ثلاث مجموعات:
-مجموعة أ: ١٥ طفل (٨ ذكور و ٧ إناث) كعينه مقارنة.
-مجموعة ب: ١٥ طفل (٨ ذكور و ٧ إناث).

-مجموعة ج: ١٥ طفل (٨ ذكور و ٧ إناث).

وقد تم تصنيع ثلاث عينات من الزبادى المحضر من اللبن الجاموسى كالتالى:

-**عينه أ**: يوجهورت حيوى غير مدعم للمجموعه المقارنة.

-**عينه ب**: يوجهورت مدعم يحتوى على٢٠مجم زنك أسيتات (٦مجم زنك)/ ٢٤٠مل لبن للمجموعة ب.

-**عينه ج**: يوجهورت مدعم يحتوى على٦٦مجم زنك أسيتات (٢٠مجم زنك) /٢٤٠مل لبن للمجموعه ج.

وقد تم اختبار الخواص الكيميائيه، الطبيعيه و الميكروبيولوجيه للعينات الثلاث. و تم إدخال اليوجهورت للوجبات مقسما على مرتين أو أربع مرات يوميا تبعا لشهيه الطفل إلى جانب العلاج الروتينى المتبع بالمستشفى مع متابعه الحالات سريريا منذ لحظة دخولها لوحده الصدر حتى يوم خروجها. و تم قياس نسبه الزنك فى مصل الدم، هيموجلوبين الدم و سى- ريأكتيف بروتين فى الدم لحظة دخول المستشفى للمجموعات الثلاث كما تم قياس نسبه الزنك فى مصل الدم وقت الخروج من المستشفى للمجموعتين (ب و ج).

وقد أسفرت النتائج عن تحسن فى مستوى الزنك فى مصل الدم و تقصير فتره المرض و تقليل حدة الأعراض فى المجموعتين ب و ج المزودين باليوجهورت المدعم بالزنك عنهم فى مجموعة المقارنة ـ أ .

التوصية: يمكن الاستناد إلى أن اليوجهورت المدعم بالزنك ساهم فى تقليل فترة التواجد الكلية بالمستشفى وتقصير فترة اعراض الإلتهاب الرئوى الحاد بالتوازى مع العلاج الروتينى للمشفى فى جرعات تتراوح بين ٦ملجم/يوم إلى الأكثر فاعليه ٢٠ملجم/يوم من الزنك كمكمل غذائى ، مما يثبت فاعليه الزنك فى التأثير على تحسين حاله الجهاز المناعى للأفراد.