Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.3, No.3, 2013

Studies on the effect of cations on antibody stimulation in rabbits

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Abstract:
Trace elements play diverse roles in maintaining body physiology but it contribution to immunity is not completely understood. This study evaluates the effect of the cations on rabbit challenged with *Salmonella enterica*. Thirty five female adult New Zealand rabbits were grouped into seven, 5 per each cation and were supplemented with 1 ml / day of single and double strength concentration of magnesium (Mg^{2+}), zinc (Zn^{2+}) and copper (Cu^{2+}) for 24 days, the control was not supplemented with any cation. All the groups were challenged with oral administration of 0.5 ml of 10^{6} CFU / ml of saline suspension of *S. enterica* on three exposures on alternative days. Cardiac blood samples were collected aseptically from the anaesthetized rabbits. The Mg, Zn, and Cu supplemented groups showed marked decreased in leucocytes counts, the effect of Mg and Zn on leucocytes count was not concentration dependant but Cu showed a concentration depended effect. The effects of Mg and Zn on lymphocyte count was concentration dependent yielding 28.3 % and 47.7 % for single and double strength of magnesium, 23.7 % and 28.9 % for single and double strength of zinc respectively. Copper and Zinc yielded double fold increase in antibody titre with a reciprocal of 16 and 32 respectively. The findings suggest an immunological enhancing effect of these cations while zinc and copper have been the most effective in influencing the humoral mechanism.
Key words: Cations supplementation, Lymphocytes count, Antibody stimulation, Immune responses

1.0 Introduction:

Salmonelloses include infection by any of the approximately 2000 serotypes of salmonella serotypes are considered members of a single species, *S. enterica*. Human infections are caused almost exclusively by *S. enterica* subsp. *enterica* of which three serotypes typhi, typhimurium and cholerasuis are predominantly isolated. Three clinical patterns of infections are recognized; enteric fever (typhoid fever) due to serotype typhi, acute enterocolitis, due to typhimurium among others and septicaemia type characterized by bacteremia and focal lesions, exemplified by infection with serotype choleraesuis. All types are transmitted by ingestion of organism, usually from contaminated food and drink (Lawrence *et al*., 2001). In spite of the great scientific strides recorded in the health sector in the century, the question of whether we have sufficient knowledge of the disease, its causes and effects so as to develop appropriate tools and interventions for its control and using them to the maximum advantage still remains.

It is well established that the lowering of the individual's immunity precedes disease conditions, that is, good health remains assured as long as the immunity is high. Various substances have been shown to have an impact on individual's immunity, such as drug, nutrition, chemicals and immunization (Chevaliar *et al*., 1996; Rahman *et al*., 2005). The disease process sets in as a consequence of the failure of the immune system to effectively and efficiently combat invading foreign or "non self" material that enter the body. Thus, as long as the basic functions of the immune system such as the combating the numerous pathogens that invades the body, through cellular or humoral responses for example antibody production and complement activation in adaptive immunity are efficiently executed, good health can be anticipated.

With a good understanding of factors that positively modulate the immune system and how to apply them, it is possible to develop appropriate tools for intervention in the cycle of human diseases. The present study focuses on the interaction of cations in the mounting an immune responses to bacterial invasion. It is hoped that the findings will enrich knowledge on the significance of trace element in public health.

2.0 Material and Methods:

Thirty five female New Zealand white rabbits were obtained from Microbiology Department Animal House, University of Benin, Benin City for this study. Stock cultures of *Salmonella enterica* were obtained from Nigeria Institute of Medical Research (NIMR), Yaba, Lagos while standard copper sulphate, zinc sulphate and magnesium sulphate were sourced from Microbiology Department store. The rabbits were divided into seven groups (Group 1 to 6) and control (group 7). Standard solutions of the cationic salts were prepared in sterile distilled water to obtain two concentrations of each cation (Mg^{2+}, Zn^{2+} and Cu^{2+}) as shown in treatment protocol (Table 1). The treatment dosage was also chosen in relation to values obtained from studies carries out elsewhere. All experimental protocols complied with NIH guidelines (NRC, 1985), as approved by the ethical and research committee, Achievers University, Owo.

2.1 Blood sample collection:

cardiac blood samples were collected after anaesthetizing the animal with chloroform and the blood samples were quickly transferred to clean, sterile plastic bottles with anticoagulant (EDTA) and plain tubes. The blood samples in plain tubes were allowed to cloth at room temperature and centrifuged at 2000 rmp for 10 minutes using a Gallenkamp bench centrifuge to separate serum from the whole blood, the sera separated were individually aspirated into sterile bijou bottles and quickly frozen at 4^{0}c until required for analysis.

2.2 Quantitative test using tube agglutination method:

2.2.1 "O – Antigen"

The O antigen was prepared according to the method outlined by Broackle (1978). 0.2 ml of pure culture of *Salmonella enterica* suspended in peptone water for 2 minutes was seeded on the surface of a Petri dish containing Desoxycholate – citrate agar (DCA) and incubated for 18 h at 37^{0}C. After incubation the bacteria growth was collected into a sterile centrifuge tube by repeated washing of the surface with sterile saline. It was then placed in water bath at 60^{0}C for $2^{1/2}$ h, then centrifuged at 1500 rmp for 30 minutes using bench – top centrifuge (Gallenkamp centrifuge, EEC) the supernatant was discarded and bacteria cells re-suspended in 100 ml of 0.3 % formalized saline. The suspension were placed in a sterile screw capped bottle and stored in a refrigerator until ready for use.

2.2.2 "H-Antigen"

1.0 ml of *Salmonella enterica* suspended in peptone water was inoculated into 20 ml of nutrient broth and incubated for 24 h at 37^{0}C at the end of the incubation equal volume of 0.5 % formalized saline was added and left at room temperature for 48 h. It was then centrifuged; the deposit was discarded while the supernatant was placed in a sterile screw capped bottle until ready for use.

2.3 Agglutination reactions:

Ten test tubes (numbers 1-10) were arranged on a rack and 0.5 ml of normal saline were pipette into all the tubes. A doubling dilution of the rabbit sera were made to obtain dilution of 1 in 2, 1 in 4, 1 in 8, 1 in 16, 1 in 32, 1 in 64, to 1 in 612, in duplicate. To one batch of the tube was added 0.5 ml of 'H' antigen preparation of the *S. enterica* while 0.5 ml of the 'O' antigen were added to the other batch of tubes. The tubes with 'O' antigen were then placed in water bath at 37 ± 1^{0}C for two hours with the water level adjusted to cover one third of the tube. The tubes with 'H' Antigen were incubated in a water bath at 50 ± 1^{0}C for two hours. The highest dilution of antiserum showing detectable agglutination was taken as the end point and called titre.

2.4 Total leucocytes count:

This test was done by withdrawing 0.02 ml of anticoagulated blood sample into 0.38 ml Turk's solution in a test tube, and then improved Neubauer counting chamber was charged with homogenized mixture using Dacic and Lewis method (Lewis *et al*., 2001). The chamber was later placed on the microscope stage and the cell in specified areas were counted using X 40 objective lens.

2.5 Differential leucocytes count:

A thin film of blood was made from the anticoagulated blood; air dried and stained using Leishman's method. The stained slides were viewed under microscope using immersion oil and x100 objective lens.

3.0 Results

Table 1 show the effects of cations on total white blood cells count and lymphocyte differential count of the rabbit studied. The rabbit's supplemented with copper showed marked decreased in total leucocytes counts of 8.5×10^{9}/ L (for single strength) and 4.2×10^{9}/ L (for double strength) compared to 12.5×10^{9}/ L for the control group. The decrease in white blood cell count was concentration dependent as doubling of copper dosage produced a twofold decrease in total white blood cell count. The decrease in white blood cells with exposure to copper was statistically significant ($P < 0.05$); the decrease in total white blood cell count among the rabbits treated with zinc and magnesium was not concentration dependant. The lymphocyte count rose from 16.8 % to 47.7 % for single and double strength of magnesium and 23.7 % and 28.9 % for single and double strength respectively. The rabbits exposed to copper showed the highest increase in lymphocytes count, recording 31.8 % and 35.7 % for single and double strength of copper. Obviously, there was leucocytopenia in the control rabbits, a typical feature of salmonellosis.

The effect of exposure to cation on antibody production of the rabbits is shown in Table 2. Exposure to single strength of magnesium yielded an increase in antibody titre with reciprocal value of 16 and 2, while, exposure to single and double strength of copper and zinc yielded an increase in antibody titre with reciprocal value of 16 and 32 respectively as against 4 for control. The increase was concentration dependent as a double concentration yielded double increase in antibody titre. The same effect was reflected in 'O' and 'H' antigens. Figure 1 show the correlation of antibody (titre) to lymphocyte count in the supplemented rabbits and the control group.

4.0 Discussion

An attempt was made in this study to determine the effects zinc, magnesium and copper on the response of rabbits to *Salmonella enterica* with a view to determine the effects of the cations on the immune functions using B - lymphocyte humoral assessment through sero-agglutination test.

This result suggest that cations could regulate total white blood cells proliferation and also enhance cell differentiation (Table 1) since there was a significant difference in lymphocyte population between the supplemented groups of rabbits and control ($P > 0.05$). It is therefore, implied that zinc, copper and magnesium at the dosage levels used in this study can check leucopoesis while enhancing cell differentiation.

The rabbits exposed to cations experienced an enhancement of their ability to produce antibodies against *Salmonella enterica.* The single and double concentration of cations raised the antibody titre of the rabbits to a 4 fold and 8 fold respectively. This finding suggests an enhancement of the humoral immune response in rabbits exposed to zinc, copper and magnesium. This finding is in tandem with the report of Nwokwnna (2001). The observation that is exposure of rabbits to zinc and copper improved immune function of the affected animals especially in the area of humoral immunity is not at variance with the reports of that have associated these cations with improving immune function (Chevalier *et al*., 1996; Turnland *et al.,* 2004; Bonham *et al*., 2002 and Troost *et al*., 2003)

The analysis of the results obtained in this study showed that exposure to zinc, copper and magnesium improved the well being of the affected rabbits; improved their ability to mount an immune response to bacterial infection and reduced the pathogenicity of salmonella. The implication is that these cations can be positive immunomodulators in the concentration range used in this study. Therefore, there is need for further studies to explore the potentials of cations both as immunomodulators in general and specifically as tools to combat bacterial infections.

Conclusion: This study show that zinc, copper and magnesium enhanced the ability of test rabbits to mount immune responses by impacting on the white blood cell and lymphocytes differentiation thereby improving the antibody stimulation in against *Salmonella enterica.* It may be possible to make these cations indices of health status and biomarker of mammalian immunity.

Conflict of Interests

The authors do not have a direct financial relationship with the commercial identity mentioned in this paper.

Acknowledgments

The authors wish to acknowledge Mr. John Ekundayo, the Laboratory technologist, Department of Microbiology, University of Benin, Benin City for providing us with the salts.

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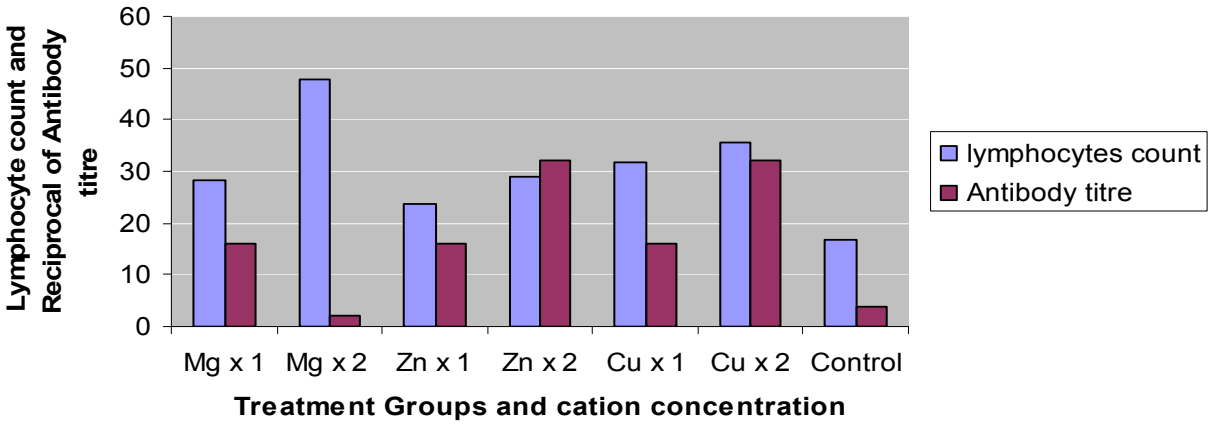
Table 1: Effects of cations on total WBC and differential count of supplemented rabbits and the control groups

Treatment groups	Total WBC count X SEM (10^9 / L)	Lymphocyte count X SEM (10^9 / L)
Mg x1	5.3 ± 0.4	1.5 ± 0.6
Mg x 2	6.5 ± 2.7	3.4 ± 0.6
Zn x 1	5.9 ± 0.2	1.4 ± 0.3
Zn x 2	5.2 ± 2.6	1.5 ± 0.7
Cu x 1	8.5 ± 0.9	2.7 ± 1.0
Cu x 2	4.2 ± 0.4	1.5 ± 1.0
Control	12.5 ± 0.5	2.1 ± 0.2

Table 2: Antibody titre (Somatic) antigen and lymphocyte count in supplemented rabbits and control group.

	Group						
Cell counts	**Mg x1**	**Mg x2**	**Zn x1**	**Zn x 2**	**Cu x1**	**Cu x 2**	**Control**
Total leucocytes count x 10^9/L	5.3	6.5	5.9	5.2	8.5	4.2	12.5
Lymphocyte count (%)	28.3	47.7	23.7	28.9	31.8	35.7	16.8
Antibody titre (reciprocal value)	16	2	16	32	16	32	4

Fig 1: Correlation of antibody (Titre) to lymphocyte count in supplemented Rabbits and control Rabbits



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