Effect of Magnetic Water on Immune Response in Rabbit against Pathogenic Bacteria

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Abstract

This study investigates the effect of drinking magnetically treated water (MTW) on animal's immune response against bacterial infection to see whether MTW improve the health and the immune response of the body, as MTW producers claimed. 32 female rabbits were housed for 58 days and divided into two groups (treated group and control group) where treated group drank magnetized water with 3500 Guse while control group drank tap water. In the twenty three day, they injected with *Salmonella typhi* somatic antigen (O-Ag) according to immunization schedule for bacteria. Coulter counter was used for measurement of innate immunity (White blood cell, lymphocyte, monocyte and granulocyte) and Architect system was used for detection of adaptive immune system (immunoglobulin M-IgM titer). No significant difference among treated group and control group was observed in innate immunity before and after vaccination with Sallmonella typhi (O-Ag). However, hemoglobin concentrations show significant decrease (p=0.027) in treated animals after vaccination with salmonella typhi O-Ag. Additionally no immunoglobulin G (IgG) was detected after 7 days from the immunization schedule, while IgM was detected and shows a significant elevation (p=0.030) in control group compared with treated group. For this reason magnetically treated water not only has no effect on hematological parameter but also has adverse effect on immune response to bacterial infection in rabbit.

Keywords*:* Magnetically treated water, Rabbit, cellular Immunity, Adaptive Immunity

تأثير المياه المغناطيسي على الاستجابة المناعية في الارنب ضد البكتريا المسببة للأمراض

الخلاصة

بحثت الدراسة التي استمرت 58 يوما تاثير المياه المعالجة مغناطيسيا على الاستجابة المناعية للحيوانات ضد الاصابة البكتيرية، من خلال متابعة التحسن في هذه الاستجابة. طبقت التجربة على

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اثنان وثلاثون انثى ارنب بعد ان قسمت لمجموعتين وهما مجموعة السيطرة ومجموعة الاختبار، جرعت مجوعة الاختبار ماء ممغنط بـ Guse 3500 بينما اعطيت مجموعة السيطرة ماء حنفية غير معامل، في اليوم الثالث والعشرين من التجربة حقنت الحيوانات بالمستضد الجسمي (O-Ag) لبكتريا *Salmonella typhi* اعتمادا على برنامج التجريع الخاص بالبكتريا، استخدم عداد Coulter لقياس عدد كريات الدم البيضاء ، واللمفاوية والاحادية والمحببة في حين ان المناعة المكتسبة تم قياسها من خلال قياس عيارية M-IgM. من النتائج لا توجد فروق معنوية بين مجموعتي السيطرة والمعاملة بالنسبة للمناعة الطبيعية قبل وبعد التجريع اضهر تركيز الهيموغلوبين انخفاضا معنويا (p=0.027) في مجموعة الاختبار بعد التجريع بالمستضد الجسمي لبكتريا *salmonella typhi* O-Ag كما لم يتم الكشف عن G (IgG) بعد اليو السابع من التجريع في حين كانت عيارية IgM معنوية (p=0.030) في مجموعة السيطرة بالمقارنة مع مجموعة الاختبار من النتائج نجد ان الماء الممغنط ليس له تاثير على المؤشرات الدمية ويسبب تاثيرات عكسية على الاستجابة المناعية في الارانب المصابة بالبكتريا.

الكلمات المرشدة: الماء المعالج مغناطيسيا، ارانب، مناعة خلوية، اصابة بكتيرية

INTRODUCTION

A Lot of research have been done to increase the quality and health benefits of water. Scientists found as soon as a permanent magnet is kept in contact with water for a significant period of time; the water gets magnetically charged and acquires magnetic properties [1], which include energy building, activating, cleansing, and detoxifying. The literature review refers that irrigation of plants with magnetized water (MW) improved the vegetative growth, yield, and yield component, fruit size and weight characters. In this regard, Selim [2] pointed out that irrigation of cereal plants (wheat, barley, and triticale) with MTW significantly affect seed germination, micronutrient, yields, and yield component. Comparable results were also reported on chick pea [3] on tomato [4], on snow pea and chickpea [5], on chili [6], and on squash plants [7].

Al-Khazan *et al.* [8] reported that MTW has an enhancing effect on the plant metabolism in terms of photosynthesis and water uptake and also have a pronounced effect on plants productivity [9].

Some studies have been done on animals. Lin and Yotvat in 1990 [10] concluded that dairy cows that drink MW have shown an increase in milk production with the same amount of milk fat in compare with cows drank non-magnetized water. Also Chicken supplied with magnetic water grew larger, with an increase in the meat to fat ratio [11]. Similar results were also recorded by [12]. Rashid *et al.* [13] reported that MTW significantly improve the titer of antibody against new castle and gumbouro disease in fawbro broiler.

Very few studies have been done to study the effect of magnetically treated water (MTW) on the performance of immune system and immune response of the animals against infection. As is well known, competent immune system is fundamental for optimal live animal's performance.

Immune response of the organism plays essential roles in the responses to different pathogens, injuries, or stressors. Innate immune system (monocytes macrophages, neutrophils, complement, cytokines, and acute phase proteins) provide

immediate host defense and plays an essential role in the early responses but often not efficiently enough to resist and eliminate complex disease conditions. While adaptive immunity is the hallmark of the immune system in higher animals and this response consists of antigen-specific reactions through T lymphocytes and B-lymphocytes and takes several days or weeks to develop [14].

The early response of macrophages and polymorphnuclear neutrophils are enough to control progression of bacterial infection and subclinical infections [15] while, T lymphocytes have the most important role in controlling the infection with intracellular pathogens through cytokine production. T lymphocytes also can mediate direct lysis of infected cells or activate B and T cells [16].

This experiment was designed to examine the effect of drinking magnetically treated water on the innate (granulocyte and monocyte) and adaptive immune response (lymphocyte, immunoglobulin M and immunoglobulin G) of rabbits against bacterial infection.

Materials and Methods

Rabbit and Housing

Thirty two (32) female rabbits weighing 1.852±0.380 Kg at the time of experiments, were housed at 25°C and divided into two groups, treated group-Drank magnetic water (n=16) and control group Drank tap water (n=16), with free access to food and water. The water in the first cages was exposed to magnetic field (3500 Gause: 24 h/day) for 58 consecutive days.

All animals were housed for two weeks before starting the experiment to exclude pregnant and sick rabbits.

Collection of Blood Sample

The animals were weighed at the first day experiment using an electronic balance (WT 15000 1B, ROHS, China). In the first thirty six days, all animals were anaesthetized, with ethylic ether, and bleed 3 times with 12 days interval before starting immunization schedule [17] and last bleeding was done 7 days after immunization schedule (Table.1) Three milliliter of blood was taken by a heart puncture using 5 ml syringe and adds to potassium-EDTA tube to determine the hematological parameters in the 1^{st} and 2^{nd} bleeding while in the 3^{rd} and last bleeding 5 milliliter of blood was taken, 2 ml of blood was added into potassium-EDTA tube for hematological parameters and 3 ml was centrifuged at 3000 rpm for 10 min to obtain the serum for detection of IgG and IgM. Bleeding was done always at the same time of the day.

Table (1). Timetable for bleeding and immunization schedule of Rabbits

Bleeding and Immunization Schedule	Treated Group (Rabbits drinking MW)	Control Group (Rabbits drinking tap water)
Experiment Start	Day 0	Day 0
1st Bleeding	Day 12	Day 12
2nd Bleeding	Day 24	Day 24
3rd Bleeding	Day 36	Day 36
Immunization Schedule 1st Injection (0.1 ml I.V.)	Day 39	Day 39
2nd Injection (0.25 ml I.V.)	Day 42	Day 42
3rd Injection (0.25 ml I.V.)	Day 44	Day 44
4th Injection (0.5 ml I.V.)	Day 47	Day 47
5th Injection (0.75 ml I.V.)	Day 49	Day 49
Last Injection (1.0 ml I.V.)	Day 51	Day 51
Last Bleeding	Day 58	Day 58

Immunization Process

Somatic "O" antigen of *Salmonella typhi* (14028 ATCC, USA) was prepared according to Myers procedure [18]. Immunization schedule was start 3 days after the third bleeding and last for 12 days. Before starting immunization schedule and one week after the last injection all the animals was bleeding and serum and blood were collected for detection of IgM and IgG.

Blood Parameters

Complete blood counts (CBC) included white blood cells (lymphocyte, monocyte, granulocyte) and hemoglobin concentrations were all measured by coulter counter (Bekman Coulter ATC "diss 2"), AOA medical electronics CO., LTD. Kobe. Japan.

Detection of IgG and Ig M

Specific antibody response to *Slmonella typhi* O antigen was detected using IgG/IgM kit (Polymed Therapeutics, Inc Houston, TX77056, USA). Then all the samples were analyzed using Architect System C8000 (Abbott-USA) for quantitation of IgM using immune turbidimetric procedure.

Statistical Analysis

Data were analyzed using SPSS software (Version 12). The results were expressed as means ± S.E and comparison of two means was made using Student's paired t-test. A value of $p \leq 0.05$ was considered to be significant.

Results and Discussions

The worldwide controversy regarding the effects of magnetic field on water results in part from the physical structure of liquid water. When water is supply with

magnetic charge; hydrogen micro clusters are formed, which react with oxygen (O_2) to generate sodium hydroxide (NaOH). As a result the water's pH is increased making it extra alkaline. Through drinking sufficient magnetic resonance water, it can rise the alkalinity of blood with a low pH of 7.2 to 7.6 [19]. Some researchers reported that magnetic treatment affects light absorbance and surface tension of water [20, 21]. While others reported that salt removal percentage (SRP) increased with increasing the current at low flow rates up to 0.75 ml/s [22]. With increasing exposed time the magnetized effects is increased, but were weakened with the removal of magnetic field. In the X-ray experiment, the strength of diffraction increased also, after the water was exposed in magnetic field. These phenomena are simply explained by the molecular structure of water and the theory of magnetization of water [23].

As illustrated in table 2, drinking magnetic water for 32 days, using paired T-test, had no significant effect on total Wight blood cells, lymphocyte, monocyte and granulocyte, and hemoglobin concentration in both groups and these findings was in agreement with Battocletti *et al*. [24] who reported that Differential and absolute white blood cell counts of segmented neutrophils and lymphocytes in rhesus monkeys were considerably changed during the test, but this change also include monkeys which were not exposed to the magnetic field. Furthermore, Gilani and his colleague [25] reported that magnetized water has no effect on some serum component such as cholesterol, triglycerides, calcium, phosphorus, and alanine aminotransferase (ALT). However magnetized water was significantly decreased aspartate aminotransferase (AST) in serum of broilers that drank magnetized water.

Furthermore, no important difference in monocyte, granulocyte, total white blood cell and lymphocyte was observed before and after vaccination with *Sallmonella typhi* O-Ag except for hemoglobin concentration, which show significant decrease in treated animals after vaccination (Table.3).

Table (2). Effect of Magnetic Water on white blood cell, Lymphocyte, Granulocyte, Monocytes and Hemoglobin

Blood Parameters	Treated Group			Control Group		
	Time 1 (Day 1)	Time 2 (Day 32)	P-Value	Time 1 (Day 1)	Time 2 (Day 32)	P-Value
W.B.C. 10^9/l	8.100 ±1.488	7.575 ±1.356	0.863	5.900 ±1.034	5.275 ±0.712	0.567
Lymphocyte10^9/l	4.000 ±0.633	4.425 ±0.892	0.771	2.700 ±0.308	2.925 ±0.487	0.614
Granulocyte 10^9/I	2.625 ±0.855	1.875 ±0.286	0.350	2.300 ±0.795	1.525 ±0.085	0.315
Monocyte10^9/l	1.475 ±0.467	1.275 ±0.209	0.766	2.475 ±1.377	0.925 ±0.165	0.376
Hemoglobin (Hb) g/dl	12.250 ±0.275	13.725 ±0.225	0.058	13.625 ±1.191	12.825 ±0.912	0.499

Table (3). Effect of magnetic water on blood parameters before and after vaccination with Salmonella typhi

Blood Parameters	Treated Group			Control Group		
	Before Vaccination	After Vaccination	P-Value	Before Vaccination	After Vaccination	P-Value
W.B.C. 10^9/l	7.575 ±1.356	6.500 ±0.544	0.321	5.275 ±0.712	5.350 ±1.252	0.955
Lymphocyte 10^9/l	4.425 ±0.892	3.075 ±0.392	0.198	2.925 ±0.487	2.575 ±0.755	0.713
Granulocyte 10^9/I	1.875 ±0.286	2.300 ±0.371	0.169	1.425 ±0.085	1.975 ±0.430	0.279
Monocyte 10^9/l	1.275 ±0.209	1.125 ±0.143	0.297	0.925 ±0.165	0.800 ±0.168	0.537
Hemoglobin (Hb) g/dl	13.725 ±0.225	11.725 ±0.402	0.027	12.825 ±0.912	13.900 ±0.234	0.332

Our finding is totally in agreement with Osbakken *et al.* [26], who reported that magnetic field has no effect on white blood cell counts, cholesterol, triglyceride, plasma creatine phosphokinase, lactic dehydrogenase, or protein concentrations in Magnet groups compared to the two control groups, However the decrease in hemoglobin concentration seems to be related to laboratory environment surrounding the magnet, when compared to the animal facility environment, quite than to magnetic field effects.

IgM is ten times more efficient than IgG in removing of *Salmonella* from blood after intravenous (IV) injection and their uptake in the reticuloendothelial system (RES). However, IgM was over 1000 times more efficient than IgG in promoting killing of the bacteria after intraperitoneal (IP) challenge. The greater effect of IgM is suggested to be a special feature of Salmonella infections, connected with the capacity of these bacteria for intracellular survival and multiplication in the RES [27].

Surprisingly, Specific antibody response (IgM) to *Slmonella typhi* (O-Ag) show significant elevation (0.030) in control female rabbit groups compared with treated group (Table 4). This indicated that magnetically treated water have adverse effect on immune system and this result was comparable to Al-Mufarrej, *et al*. [1] who show that magnetic treated water have adverse effect on female broiler chickens immune response and these result is also comparable to Tenford *et al.* [28] who show that humoral and cell-mediated immune responses, including the level of serum IgM and spleen lymphocytes, to sheep erythrocytes vaccination were not significantly different for the exposed mice in comparison with control animals.

Table (4). Effect of magnetic water on Immunoglobulin M (IgM) titer after Immunization with Salmonella typhi

Groups	IgM titer (g/l) (Before Immunization)	IgM titer (g/l) (After Immunization)	P-Value
Control Group	0.345 ±0.009	0.580 ±0.066	0.030
Treated Group	0.310 ±0.021	0.390 ±0.046	0.078

Eventually some magnetic water treatment effect actually exists, but the most important questions is whether the magnetic water improve the health and the immune response of the body as magnetically treated water producers claimed? It seems quite unlikely that any of the claimed benefits of MTW are real until such data become available. Furthermore, there are other significant factors which can affect the outcome of MTW and should be examined before any final conclusion concerning such action. These contain factors influence the strength of the magnetic field for example the number of magnets, space between magnets, shape of the magnet, the space between the water and the magnetic field, quantity of water and finally the period of contact with the magnetic field [1].

Conclusions

MTW did not only not influence the innate immune response, adaptive immune response, but also inhibit primary immune response (IgM) against bacterial infection. A little scientific work was done in the field of effect of MTW in vivo to prove the positive effect of MTW to support the claims made by the supplier of magnetic devices. Finally, it's very important to develop the knowledge of magnetic water treatment the point where the effects of magnetic treatment can be reliably predicted and shown to be economically attractive.

References

[1] Al-Mufarrej S, Al-Batshan HA, Shalaby MI, and Shafey TM. "The Effects of Magnetically Treated Water on the Performance and Immune System of Broiler Chickens", International Journal of Poultry Science, 4: 96-102, 2005.

[2] Selim MM. The 3rd International Conference on Water Resources and Arid Environments and1st Arab Water Forum. King Saud University. King Fahd Cultural Center in Riyadh, Saudi Arabia, 2008.

[3] Hozyan M and Abdul-Qados MS. "Irrigation with magnetized water enhances growth, chemical constituents and yield of chickpea", (Cicer arietinum L.). Agriculture and Biology Journal of North America, 1: 671-676, 2010.

[4] Abou El-Yazied AE, Shalaby OA, El-Gizawy AM, Khalf SM and El-Satar A. "Effect of magnetic field on seed germination and transplant growth of tomato", Journal of American Sciences, 7: 306-312, 2011.

[5] Grewal HS and Maheshwari BL. "Magnetic treatment of irrigation water and snow pea and chickpea seeds enhances early growth and nutrient contents of seedlings", Bioelectromagnetics, 32: 58-65, 2011.

[6] Datir RB, Apparoo BJ, and Laware SL. "Application of amino acids chelated micronutrients for enhancing growth and productivity in chili (Capsicum annum L.)", Plant Scinences Feed, 2: 100-105, 2012.
[7] Abd El-All HM, Ali SM and Shahin SM. "Improvement growth, yield and quality of squash (Cucurbita pepo L.) Plant under salinity conditions by magnetized water, amino acids and selenium", Journal of Applied Sciences Research, 9: 937-944, 2013.
[8] Al-Khazan, M, Abdullatif BM, and Al-Assaf N. "Effects of magnetically treated water on water status, chlorophyll pigments and some elements content of Jojoba (Simmondsia chinensis L.) At different growth stages", African Journal of Environmental Science and Technology, 5: 722-731, 2013.
[9] Basant LM, Harsharn SG. "Magnetic treatment of irrigation water: Its effects on vegetable crop yield and water productivity", Agricultural Water Management, 96: 1229-1236, 2009.
[10] Lin IJ and Yotvat J. " Exposure of irrigation and drinking water to magnatic field with controlled power and direction" Journal of Magntism and Magnetic Materials, 83: 525-526, 1990.
[11] Gholizadeh M, Arabshahi H, Saeidi MR, and Mahdavi B. "The effect of magnatic water on growth and quality improvement of poultry", Middle-East Journal of Scientific Research, 3: 140-144, 2009.
[12]Shamsaldain QZ and Al Rawee EA. "Effect of magnetic water on productive efficiency of Awassi sheep", Iraqi Journal of Veterinary Science. 2: 129-135, 2012.
[13] Rashid KA, Al-Hillali AHK, Neda SM, and Irhaim KI. "Effect of Magnetically Treat Water on Immunological Response in Fawbro Broiler", Iraqi Journal of Veterinary Science .4: 17-23, 2009.
[14] Parkin A and Cohen B. "An overview of the immune system", The Lancet (ISO4), 357: 1777-1789, 2011.
[15] Hughes EA and Galan JE. "Immune Response to Salmonella: Minireview Location, Location, Location", Immunity, 16: 325–328, 2002.
[16] Adrianus W. M. van der Velden, A.W.M.; Copass, M.K. and Starnbach, M.N. "Salmonella inhibit T cell proliferation by a direct, contact-dependent immunosuppressive effect", PNAS. vol. 102 (49) 17769–17774, 2005.
[17] Burrel, R. "Experimental Immunology", Fifth Edition. Burgess Publishing Company, USA, 1979.
[18] Myers RH. "Immunology- A Laboratory Manual. Second Ed", Wm.C. Brown Publishers, USA, pp.3-5.1995.
[19] Lam M. "Magnetic Water", (www.DrLam.com), 2001.
[20] Joshi KM and Kamat PV. "Effect of magnetic field on the physical properties of water", Journal of Indian Chemical Society, 43: 620-622.1966.
[21] Bruns SA, Klassen VI, and Konshina AK. "Change in the extinction of light by water after treatment in a magnetic field", Kolloidnyi Zhurnal, 28: 153-155. "
[22] AbdelHady RS, Younes MA, Ibrahim AM, AbdelAziz, MM. "An empirical model for salt removal percentage in water under the effect of different current intensities of current carrying coil at different flow rates", Journal of Advanced Research, 2: 351–355, 2011.
[23] Deng B, and Pang X. "Variations of optic properties of water under action of static magnetic field", Chinese Science Bulletin, 52: 3179-3182, 2007.
[24] Battocletti JH, Salles-Cunha S, Halbach RE, Nelson J, Sances A JR, Antonich FJ. "Expo sure of rhesus monkeys to 20 000 G steady magnetic field: effect on blood parameters", Medical Physics, 8:115-118, 2001.

[25] Gilani A, Kermanshahi H, Golian A, Gholizadeh M, Mohammadpour AA. "Assessment of magnetized drinking water on excreta quality, nutrients digestibility, serum components and histomorphology of digestive tract in broiler chickens", Research Opinions in Animal and Veterinary Sciences, 4: 120-127, 2104.
[26] Osbakken M, Griffith J, and Taczanowsky P. "Agross morphologic, histologic, hematologic, and blood chemistry study of adult and neonatal mice chronically exposed to high magnetic fields", Magnetic Resonance in Medicine, 3: 502-517, 1986.
[27] Saxen, H. "Mechanism of the Protective Action of Anti-Salmonella IgM in Experimental Mouse Salmonellosis", Journal of General Microbiology, 130: 2277-2283, 1984.
[28] Tenfords TS and Shifrine M. "Assessment of the responsiveness of mice exposed to a 1.5 Tesla stationary magnetic field", Bioelectromagnetics, 5: 443-446, 1984.