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A STUDY ON IMPROVING MUMPS VIRUS VACCINE BY SUPPLEMENTING DIET WITH *STELLARIA MEDIA*

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ABSTRACT
In last few years, the incidence of mumps reinfections have elevated in immunized individuals in Iraq for unknown reasons are not clear. The use of herbs with the vaccine for boosting the immune response could be a good trial to improve the vaccine's protection from the infection. An animal model is used in this study to evaluate the potency of mumps virus vaccine and the boosting effect of *Stellaria media* supplementation diet, to generate a higher immune response in mumps vaccinated mice. The present study results give an idea to get benefit of using *Stellaria media* extract at the time of vaccination in order to improve the immune response to mumps vaccine.

KEYWORDS: Improve, Mumps, Vaccine, Stellaria.

INTRODUCTION

Mumps is the viral infection which causes high morbidity in populations before vaccine introduction. Also this viral infection can cause many disorders for example meningitis, orchitis and deafness in infected persons [1]. After mumps vaccines have been introduced and systematically using, the incidence of mumps infection is reduced. In spite of that the recurrence mumps infection outbreaks in some countries has increase awareness of mumps disease and its vaccine protection. In the initial success of vaccination to control the infection, the elimination of mumps may be hard to achieve [2]. To measures the immune response against mumps infection after immunization, it is important to know if the vaccinated individual becomes susceptible or not to the infection, but measuring the mumps-specific antibodies if it reaches a low level or become no more detectable. The titers of antibodies production of plasma cells are the markers to measure vaccine potency, in spite of that the vaccine-induced immunization is mediated by many effectors of the adaptive immunity, represented by T helper cells and cytotoxic T lymphocytes. The protection provided by vaccine result in immediate protection and also immunity against infection in the future due to the formation of B and T memory cells. Studies on measuring antibody titers against mumps after weeks and years' post vaccination in the first trails, the seronegative babies developed high sero conversion more than 90% after vaccination[3]. However, some post-marketing reports have revealed the fact that the mumps infections still a problem with time after vaccination andmaycause outbreaks of mumps [4]. Many studies on vaccinated persons that received two doses of MMR vaccine, blood samples were taken after 5 to 15 years. The studies showed declines in the titer of anti-mumps with the time, also a decrease in the proportion of individuals that have detectable specific antibodies were observed [5].

Mumps infection cause over 90% sero conversion in the population at the age of 14 years before vaccine was given[6] but some infections is occured, this can because severe complications in central nervous system [7]. Mumps virus is a belongs member of *Paramyxoviridae*. The virus genetic material is anon segmented -ssRNA containing 15,384 nucleotides. The genome encodes for six structural proteins and two accessory protein mumps viruses' subtypes are twelve from A to L which classified according to the hydrophobic gene sequence [8]. Mumps disease can be prevented by vaccination. The vaccine licened firstly in United States of Amerika in 1967. The vaccine was monovalent then substituted with a trivalent vaccine known as MMR, containing vaccines for measles, mumps and rubella. The infection is decrease after the introduction of very potent mumps vaccine which effectively reduced the incidence of mumps infections [6]. Although of continuous vaccination within populations in most countries, occurrences of some mumps disease cases or even outbreaks take place; such as outbreak happened in Iowa in 2006 [9] have raised many questions regarding the pathogenesis of mumps vaccine efficacy. Studied began using animal models determine the reasons of eased these outbreaks, for example, mice, as low cost animals present ideal models for many human infectious pathogens [10]. The fibroblast cell of mouse adapted mumps virus was showed viral replication in the lungs. Further studies used mice to investigate the immune status after vaccination by live attenuated strains of the virus [11,12]. In a study it was found that mice stimulate the cellular and humoral immunity to Mumps infection [13]. Most of the studies employing herbal medicine assessed the effects of these supplements on the vaccination-immune response outcome. Stellaria media is best known for its ability to cool inflammation and speed healing for internal or external flare-ups. It is widespread in North America, Europe and Asia. *Stellaria media* is edible and nutritious and is used as a leaf vegetable, often raw in salads [14]. This study was achieved for investigation the effects of *Stellaria media* extract on the protection outcome resulted after a Mumps vaccination.

MATERIALS & METHODS

Plant collection

Stellaria media were collected from Baghdad University. The leaves were air dried in the shed.

Extraction Procedure

The air dried leaves were powdered. A conventional extraction was done; 50 ml of distilled water was added to 5 g of powdered leaves material in a round bottom flask then refluxed for 5 h at 100 °C. Liquid extracts were separated from the solid residue using vacuum filtration and concentrated using rotary evaporator [15].

***Staphylococcus aureus*:**

The bacterial strain was provided kindly by biology department laboratories, college of science, Baghdad University. The bacterial suspension was prepared in a concentration of 110^8×cell/ml.

Haemagglutination Ag:

The antigen was prepared from Mumps vaccine which was provided by WHO, kept in 4◦C until used.

Alsever's solution

It was prepared from of 2.05%dextrose, 0.8% sodium citrate, 0.055% citric acid, and 0.42% sodium chloride.

Phosphate buffered saline (PBS):

One tablet of PBS in 100 ml D.W. and sterilized in the autoclave (121º C for 15 minutes), PH=7.4. 0.1% Bovine serum albumin. The solution preparation was kept in 4◦C.

Kaolin (Aluminum silicate), PH= 8.6. Was prepared in two dilutions: Suspension of 50% and Suspension of 0.75%.

Methods

Eight mice which with approximately 250 g weight were used in this study four of them were immunized with 0.1 ml Mumps vaccine and their nutrition was supplemented with *Stellaria media* extract during the experiment period. Two mice have immunized diet didn't supplement with the herb and two mice didn't immunize as a control. Blood samples were taken before vaccination, then after 3 weeks of vaccination blood samples were collected for evaluation of immune system after immunization with Mumps antigen. Phagocytosis in vitro was done, Mumps-specific antibodies were measured by haemagglutination inhibition test and W.B.C count was done.

Blood Samples Collection:

Blood was collected by heart puncture using a syringe, then 0.5 ml the blood was distributed into the first container with anticoagulant (heparin). These samples were used to calculate W.B.Cs. count and the percentage of the efficiency of phagocytic cells to evaluate the immune response. The test was done within 1 hours of blood collection. The remainder of the samples were added to tubes free of any preservative and incubated at 37 °C for a quarter of an hour to complete the clotting process and then placed in the refrigerator for two hours. The tubes were centrifuged 2000 r/ min for ten minutes, and then the serum was collected and transferred to another sterile tube for heamagglutination inhibition test.

1.**Counting white blood cells** (W.B.C.): The test was performed using Neubauer chamber.

2. **PhagocytosisTest :**

This test was done to determine phagocytosis index done according to Furth *et al.*, 1985 [16]. The phagocytosis index was determined before and after immunization with Mumps vaccine for each experimental and control animal. Phagocytosis index was calculated by counting 100 phagocytic and non-phagocytic cells.

$$\text{Phagocytosis index} : \frac{\text{no.of phagocytic cells}}{\text{100 phagocytica+ non- phagocytic cells}} \times 100$$

3. Hemagglutination Inhibition (HI):

Red blood cells from a human with (O) blood group were collected in Alsever's solution.

Haemagglutination (HA)

A volume of 25µl from PBS was added to each well then Mumps virus was put in the first well in a volume 25µl, two fold dilutions were made leaving the control well. Equal volume of PBS was added to all wells, then the same volume of 0.5% RBCs suspension was added, the components were mixed, the results were read after an hour.

HAI test

For detection of the specific antibodies to the viral heamagglutination by mumps, using human O red blood cells. The RBCs were washed and then suspended in a final concentration of 1% in PBS buffer. The assay was done using the method of Stephenson *et al.* [17]. The HAI titer of the each serum sample was determined as the inverse of the last dilution where agglutination not occurs.

RESULTS & DISCUSSION

The current study was done to evaluate the effects of *Stellaria media* extract on the immune response to mumps vaccine. The protection outcome evaluated is by antibody production determination, phagocytosis index calculation and W.B.Cs counting. The study results revealed that the mice subjected to the mumps vaccine demonstrated a high increase in mumps specific antibody titer, phagocytosis index and W.B.Cs count after 3 weeks of immunization in 2 and 3 groups compared to the control. Also, the results show a greater immune response in group 3 compared to group 2. These results suggest that vaccination is more efficient when introduced with supplementation the nutrition of subjects with *Stellaria media* extracts as shown in table 1. Vaccination stimulates innate immune system which results in an inflammatory response. That is essential to control the infections before the infections can spread through the body. The innate immunity activates the adaptive immune system [18] . The phagocytes have an important role in acute inflammation due to their efficiency in engulfment and destruction of a variety of pathogens. Phagocytes cells include neutrophils, macrophages, monocytes and eosinophils. They are called professional phagocytes. Phagocytosis is the key element of host defenses against many infections[19]. Polymorphonuclear cells (PMN) serve as the first line of the cellular immune response. PMN exhibit a ruffled surface of pseudopods on their surface, which increases the surface area of the cells and improve phagocytosis by increasing phagosomes formation [18].

TABLE 1: The average values of W.B.Cs Count, Phagocytosis Index and Haemagglutination Inhibition of Mumps antibody concentrations within the experimental groups:

Groups	1	2	3
No. of W.B.Cs	4 .3 × 10^3	6.5 × 10^3	10.6 × 10^3
Phagocytosis Index%	0.06	0.62	0.78
HI titer of IgM Mumps Abs in IU/ml	0	8	16

Group 1: Control group
Group 2: Mice immunized with Mumps vaccine group.
Group 3: Group of Mumps immunized with Measles vaccine and theirDietwassupplemented with *Stellaria media*extract.

Many studies were done to investigate the immune responses after routine the trivalent MMR vaccine introduction to one year-old infants, lymphoproliferation in response to antigens and NK activity were unchanged or improved after immunization. In others, unexpected differences in the immune response of Bedouin and Jewish children receiving the same vaccine were result. After vaccination, Bedouin infants had higher WBCs, higher levels of IgG [19].

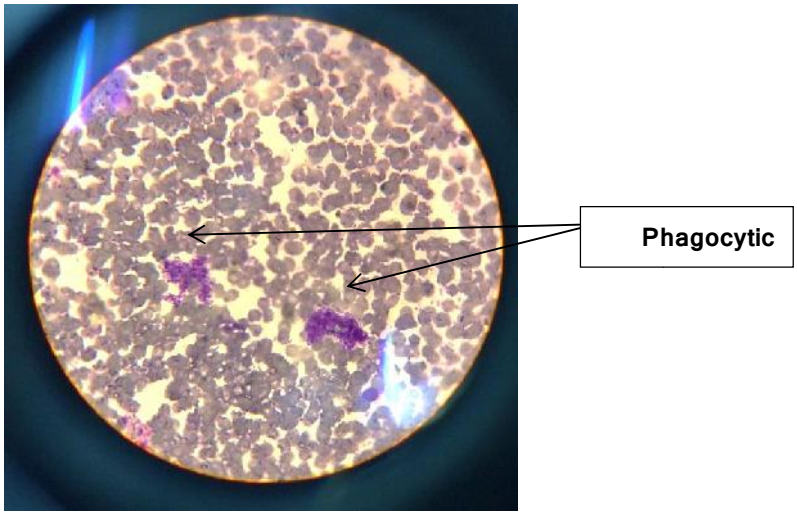

IMAGE 1: Phagocytic cells (Neutrophil) engulf *Staphylococcus aureus*

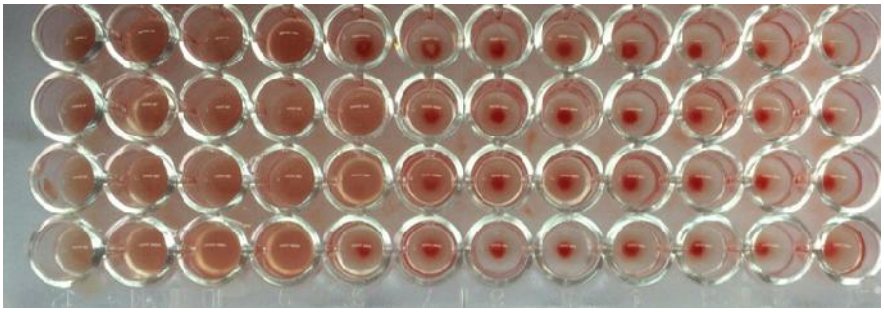

IMAGE 2: One microtiter plate represents Haemagglutination Inhibition test

Herbal medicine products claim to have beneficial effects on boosting or restoring animal health parameters in stressful and diseased conditions. 0ne investigation study that employed a mannan-oligosaccharide as an additive to the diet of hens, the results revealedimprovements in egg quality and survive ability under heat stress on weight gain. The herbal supplementation additives stimulate the humoral immune response [20]. Another experiment results showed that the herbal extract of oregano, laurel leaf, and lavender oil mixture may help in decreasing the deleterious effects of some parasites on the production performance of chicks [21].

This study is done to estimate the boosting effects of *Stellaria media* on immune response to Mumps vaccination. The results show that the introduction of *Stellaria media* as a herbal supplement, promote improvement to immune systems leading to successful immunization outcomes presented in elevation in numbers of white blood cells, phagocytosis index and generated robust anti-Mumps antibody responses. The current study recommendation is Addition of *Stellaria media* extract feed supplemented could be useful and can improve munps vaccine potency within the population.

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