

EFFECTIVENESS OF CORIANDER SEEDSON IMMUNOLOGICAL PARAMETER IN IRAQI AWASSI EWESVACCINATED WITH *Brucella* REV-1 VACCINE

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ABSTRACT

A study was conducted to focus on the immunomodulatory effect of different concentrations of Coriander seeds as feed additive on some immunological parameters in Iraqi Awassi ewes vaccinated with *Brucella* Rev-1 vaccine. The study was conducted on eighteen ewes of 2-3 years of age were randomly divided in equal number to three groups (6 each) body weight was considered. Ewe in first group kept as control and were daily fed on concentrate diet (350 g/head) for one month, were as thesecond groupwas fed as in Ist group with the diet contain(2.5%) coriander seeds and third group was fed the same diet contain(5%) of coriander seeds treated with the *Brucella* Rev-1 vaccine, third group: treated were vaccinated with *Brucella* Rev-1after a period of treatment). allEwes groups were vaccinated with brucella Rev-1 vaccine on day 1st day after treated diet experiment for month, blood samples were taken from ewesand tested as follows(7,14,21 and 28days)after vaccination for calculating of phagocytic activityindex percentage in peripheral blood by nitro blue tetrazolium test and anti-*Brucella* antibody level in serum by ELISA assay. Results revealed that second & third groups were significantly ($P \leq 0.05$) recorded higher values in their peripheral blood mitotic index, phagocytic activity by nitro blue tetrazolium test and anti-*Brucella* antibody titer level in serum by ELISA assay as compared with the control group . Third group Ewes results were revealed significantly($P \leq 0.05$)increased in phagocytic activity index of peripheral blood by nitro blue tetrazolium test and anti-*Brucella* antibody titer level in serum estimated by ELISA as compared with those in second group. The result revealedimprovement of non-specific and humoral immunityof Coriander seeds treated ewes and vaccinated with *Brucella* Rev-1 as compared with un treated animals. Present study results reported that Coriander seeds demonstrated a pronounced immunopotentiators effectthrough the higher enhancement of all immunological parameters that carried out in this experiment.

INTRODUCTION

Immunomodulators are biological materials that mediate the effectors mechanisms of the immune system through immunostimulation to a given antigens or potentiate the effectiveness of a vaccine (1). Scientists have begun to adopt vaccine strategies that are based on the magnification of antigen presentation for major histocompatibility complex (MHC) class I or class II molecules due to the importance of these molecules in immune response, especially those materials that act as immunomodulators (2). The plant extracts, derivatives or their by-products, have also been the interest of investigators as immunomodulators to overcome the disadvantage of biological and chemical immunomodulators (3). One of these plants is *Acoriandrum sativum*. *Acoriandrum sativum* (coriander) which is considered as herb and spice. Its leaves & seeds are used as seasoning condiment. Coriander seeds have health supporting reputation which is listed as one of the higher curing spices, it has been traditionally referred as antidiabetic (4), anti-inflammatory and cholesterol lowering (5). In addition, it's also used as carminative, diuretic, stimulant, stomachic, refrigerant, aphrodisiac, analgesic (6), antihelminthic (7) and hypoglycemic (8). A seed of *C. sativum* contains 0.5-1 % essential oil and are rich in beneficial phytonutrients including carvone, geraniol, limonene, borneol, camphor, elemol and linalool. Coriander's flavonoids include quercetin, kaempferol, rhamnetin and epigenin. It also contains active phenolic acid compounds including caffeic chlorogenic acid. Research also suggests that the volatile oils found in the leaves of *C. sativum* plant may possess antimicrobial properties against food borne pathogens such as *Salmonella* species (9). Prevention of brucellosis in human still depends on the eradication or control of the disease in animal hosts. The exercise of hygienic precautions to limit exposure to infection through occupational activities, and the effective heating of dairy products and other potentially contaminated foods, but vaccination may play important role in the prevention of human disease (10). Various vaccine preparations have been employed; including live attenuated *B. abortus* strains 19-BA and 104M, the phenol-insoluble peptidoglycan vaccine and the polysaccharide-protein vaccine. All had limited efficiency (11). The live vaccines have provoked unacceptable reactions in individuals sensitized by previous exposure to *Brucella* or if inadvertently administered by subcutaneous rather than percutaneous injection. These will probably require a combination of detoxified Lipopolysaccharide-protein conjugate and protein

antigens such as the L7/L12 ribosomal proteins presented in an adjuvant or delivery system favoring a T_H1 type immune response (12). This vaccine has been proved inferior to the Rev.1 strain for the prevention of *B. melitensis* infection in sheep & goats and ineffective against *B. ovis* infection in sheep. *B. abortus* strain 19 still appears to be as effective as any for the prevention of *B. abortus* infection in cattle. Further researchers used sub cellular molecules of *B. abortus* and *B. melitensis*. (13) and (14). The immunologists are engaged to design vaccine strategies to maximize the activation of the immune system and to avoid the unfavorable complications resulted from *Brucella* vaccines. One of these strategies is the employment of immune modulators to potentiate the immune response and increase the effectiveness of vaccines (15, 16 and 17). The study was conducted to evaluate the potential effect of Acoriandrum seed on some immunological parameters of Awassi ewes vaccinated with Brucella Rev.1 vaccine.

MATERIALS AND METHODS

The study was conducted on eighteen ewes at the beginning of experiment their age were ranged (2-3) years were randomly divided into equal number of three groups (6 each) body weight was considered. They were housed in bio-clean place at 20-25°C. They were freely accessed to feed and water, all animals were allowed to graze for 3-6 h/day. Before carrying out the experiments, Ewes were separately housed for a week to be experienced for acclimatization. The dried lyophilized brucella Rev-1 vaccine was supplied by the central veterinary laboratory Baghdad/Iraq. Ewe of **1st** group kept as control were daily fed on concentrate diet (350 g/head) for a month, while **2nd** group was fed same as in 1st group diet with (2.5%) coriander seeds and **3rd** group was fed same as in 1st group diet with (5%) of coriander seeds. All ewes groups were vaccinated with brucella Rev-1 vaccine supplied by whom on the 1st day after carrying out experimental diets for a month. Blood samples were taken from jugular vein weekly on the days (7, 14, 21 and 28) after vaccination time for mitotic index phagocytic cells of peripheral blood by nitro blue tetrazolium test and anti-*Brucella* antibody level in serum by ELISA assay. Kits were used during the experiments of the study was sheep serum-IFN- γ ELISA quantitative determination (Bender Med. Systems, Austria). The procedure of Chauhan 1998 (18) was followed to assess the mitotic index of peripheral blood lymphocytes of all ewes at (7, 14, and 21 days), NBT Nitro blue tetrazolium kit was supplied from (Sigma, USA). The NBT index was assessed using the following:

$$\text{equation: NBT Index (\%)} = \left(\frac{\text{Number of Positive Nutrophils}}{\text{Total Number of Neutrophils}} \right) \times 100$$

A further estimation was also given; it was treated efficiently, which was calculated according to the following equation Perez-Serrano (19):

$$\text{Treatment efficiency (\%)} = \left(\frac{A - B}{B} \right) \times 100$$

A = Treated groups
B = Negative control group.

RESULT AND DISCUSSION

Results of NBT index percentage were given in (table 1), and the treatment efficiency of each group for treated ewes was presented in (figure 1). The vaccinated ewes with Brucella-Rev-1 and treated with Coriander seeds showed different significant increases ($P \leq 0.01$) in peripheral blood NBT index percentage as compared with (control group) and a highest percentage was observed in 3rd group (28%) after (7 days) of vaccination were as the lowest percentage was founded in 2nd group (9%) at (21 and 28 days) after vaccination. The best treatment efficiency was recorded in 3rd group (133.3%) at (7 days) after vaccination were as the lowest percentage was observed in 2nd group (77.7%) at (28 days) after vaccination. Results of NBT index are showed a significantly ($P \leq 0.05$) increased percentage in treated ewes, are also in favour of such agreement, Nitro blue tetrazolium reduction by polymorphonuclear cells may requires an oxidative metabolism by the hexose monophosphate shunt, and is impermeable to cell membrane, and for it enters the cell during the process of phagocytosis, and its reduced by diphorase activity within phagosome (16). Attachment of the phagocyte to the organism is an important interaction, which may determines whether the uptake is subsequently occurs or not, and whether the killing mechanisms are triggered or not. The attachment can be mediated by two entities, lectins on the organism and lectins on the phagocyte. Of a particular interest in this aspect are the complement receptors CR3, P150, and the related molecule LFA-1, which have multiple binding sites specific for different carbohydrate moieties (10). They can bind to *B-glucans* and to the lipopolysaccharide endotoxin of Gram-negative bacteria and this can lead to complement deposition through alternative or classical pathways (14). Although macrophages and Monocytes possess engulf

mechanisms in the resting state, these mechanisms can be enhanced, and new mechanisms can be expressed when they are activated. Activation can occur through exposure to microbial products (brucellaRev-1 vaccine) and/or materials extracted from plants coriander seed. Such scan is enhanced by the findings of the present study and also confirmed by other investigators (8, 19, 20, 21 and 22). Such immunomodulators can cause a direct activation of phagocytes, or indirect activation through triggering cytokine release from them.). For optimal expression of this mechanism, macrophages need both activation by IFN- γ and triggering by TNF-alpha, and this mechanism enables murine macrophages to kill mycobacterium and probably other intracellular bacteria like *Brucella* (15).

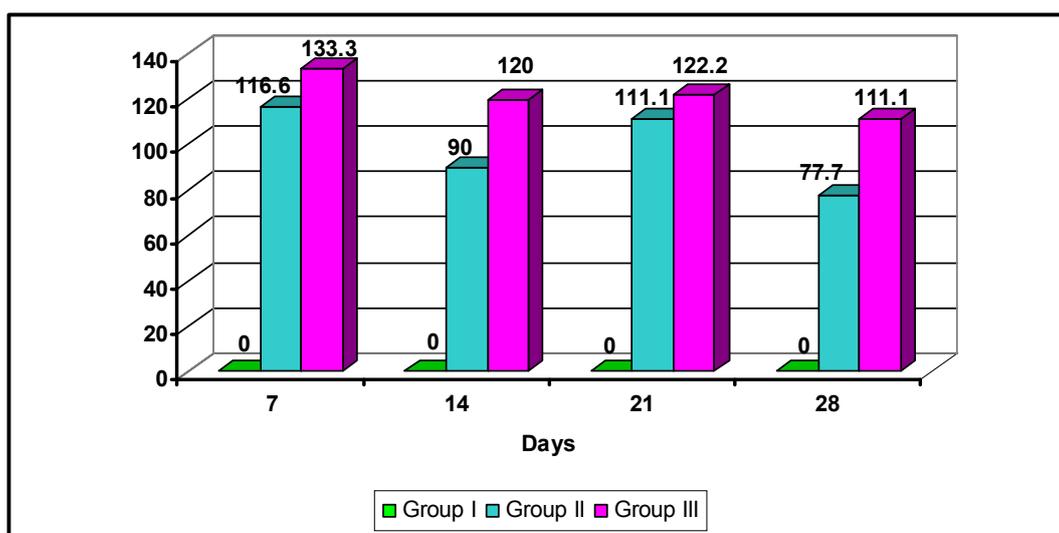
Table 1:Peripheral blood Mitotic index after vaccination

Groups	Blood Mitotic index (mean \pm S.E.: %)*			
	Day 7	Day 14	Day 21	Day 28
I	12.00 \pm 0.58 ^c	10.00 \pm 0.1 ^{ab} 0.58 ^a	9.03 \pm 0.09 ^a	9.03 \pm 0.09 ^a
II	26.00 \pm 0.58 ^c	19.58 \pm 0.3 ^{ab} 0.58 ^a	19.83 \pm 0.53 ^a	16.83 \pm 0.53 ^a
III	28.00 \pm 1.15 ^c	22.00 \pm 0.4 ^a 0.58 ^a	20.83 \pm 0.09 ^a	19.83 \pm 0.09 ^a

*Different letters: Significant difference (P \leq 0.05) between means of the same column.

21.00	18.67
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Figure 1: Treatment efficiency for Peripheral blood Mitotic index after vaccination



A similar outcome was demonstrated a significant ($P \leq 0.05$ $P \leq 0.01$) increase in the level of serum antibrucella antibodies in treated and vaccinated ewes with coriander seeds, the highest level was observed in 3rd group (61.96) on 21,28 days after vaccination and the lowest level was recorded in 2nd group (16) at 7 days after vaccination, where the highest treatment efficiency was recorded in 3rd group (84.8%) on 21,28 days after vaccination and the lowest treatment efficiency was appeared in 2nd group II (18.8%).

Table 2: Antibrucella antibodies level in sheep vaccinated with *Brucella* Rev-1 vaccine evaluated with ELIZA.

Groups	Antibrucella antibodies Serum Level (mean \pm S.E.; pg/ml)*			
	Day 7	Day 14	Day 21	Day 28
I	16.33 \pm 23.40	34.67 \pm 4.4	33.67 \pm 4.40 ^c	33.67 \pm 4.40 ^c
II	19.00 \pm 14.00	47.67 \pm 2.85	57.67 \pm 2.85 ^{ab}	57.67 \pm 2.85 ^{ab}
III	20.67 \pm 14.70	60.00 \pm 11.53	61.96 \pm 11.53 ^c	61.96 \pm 11.53 ^c

*Different letters: Significant difference ($P \leq 0.05$) between means of the same column.

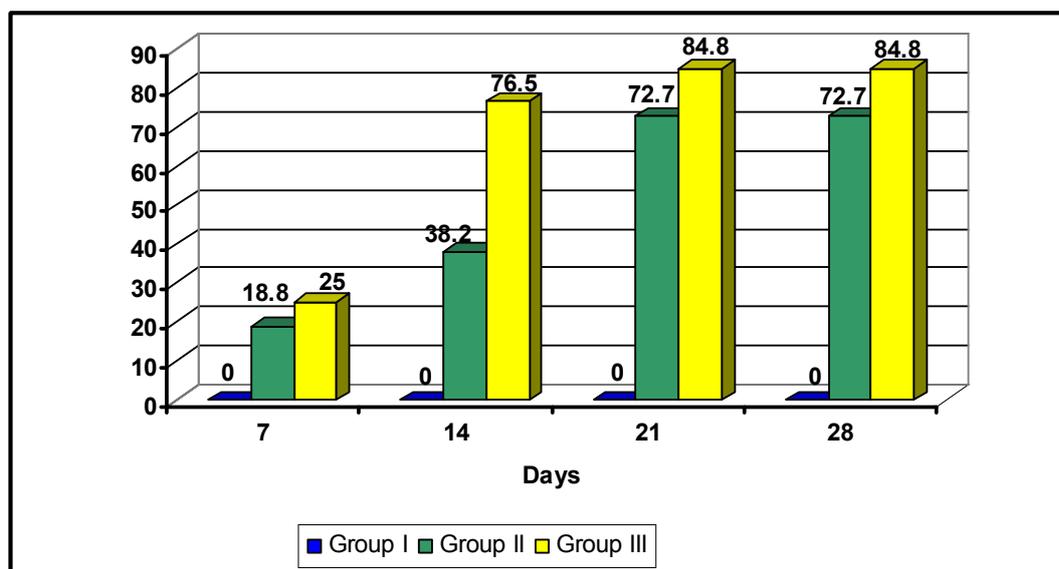


Figure 2: Antibrucella antibodies level in sheep vaccinated with *Brucella* Rev-1 vaccine evaluated with ELIZA.

Anti-brucelline antibodies showed an increased titer in all immunized groups with BrucellaRev-1 vaccine, especially groups treated with the Coriander seeds used in the study. Such observation suggests that the immunostimulation also involved the humoral immune response, although the pathway may be through the modulation of macrophages and T lymphocytes as both types of cells are required to enhance the B lymphocytes to produce immunoglobulin (1). Such findings came to confirm previous results reported by (6, 7, 9, 16 and 21). A pronounced immunopotentiators role of coriander seed were given with the Brucella-Rev-1 vaccine may be due the biochemical effect of essential oil component of Coriander seeds on mitochondria which showed a concentration –dependent inhibition in complex I and II activities of the respiratory chain, and a time-dependent decrease in ATP level. In addition, a time – dependent decrease in glutathione (GSH) level and in the reduction of nitroblue tetrazolium which was obtained, indicating an increase in reactive oxygen species (ROS) generation (20). Also other researchers reported that aqueous extract of coriander seeds showed significant inhibitory activity against inflammatory bowel disease in mice (19) while oral administration of the leaves extract at a dose of (200 mg/kg)body weight significantly ($P \leq 0.05$) reduced the toxic effect of carbon tetrachloride-induced hepatotoxicity in rat through significantly lowered (SGOT, SGPT, ACP) and increased hepatic enzyme like (SOD, CAT, TBARS) levels and increases the inflammatory cell infiltration (22), and the compounds aliphatic 2E-alkenals and alkanals, isolated from the fresh leaves of *C. sativum* were found to possess bactericidal activity against *Salmonella choleraesuis* (9). Several mechanisms of potential antioxidant activity of coriander seeds component, including determining relative free radical-scavenging and ferrous ion-chelating activities, the reducing power were examined that exhibited a significantly ($P \leq 0.05$) greater radical –scavenging activity towards both lipid and water-soluble radicals, which was attributed to the total phenolic content, bacterial cell damage resulting in significant ($P \leq 0.05$) greater growth inhibition of *B. Subtilis* and *E. coli* was observed (21), other investigators thought that the lipid and antioxidant like sterol and Tocopherol acts as free radical-scavenging (23), and increasing the leukotriene as a chemotaxis for leukocytes (24), or may be through the stimulation of the bone marrow and thymus for leukocytes production (25), the coriander seeds immunopotentiated the specific immune response through the Tocopherol which compound increases the T and B cells (26).

تأثير إضافة تركيزين مختلفين من بدور نبات الكزبرة *Coriander seeds* كإضافات علفية في فترات زمنية متفاوتة على الاستجابة المناعية للنعاج العواسية الملقحة بلقاح البروسيل

العترة *Brucella Rev-1*

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الخلاصة

درس تأثير إضافة تركيزين مختلفين من بدور نبات الكزبرة *Coriander seeds* كإضافات علفية في فترات زمنية متفاوتة على الاستجابة المناعية للنعاج العواسية الملقحة بلقاح البروسيل العترة *Brucella Rev-1*. أجريت هذه الدراسة في الحقل الحيواني في كلية الطب البيطري - جامعة بغداد للفترة من 2010/3/1 ولغاية 2010/6/31 لقياس مستوى الاستجابة المناعية كل أسبوع ولمدة 28 يوم، استخدمت ثلاث مجاميع من النعاج العواسية الناضجة بعمر (2-3) سنة قسمت عشوائياً بواقع (6) نعاج في كل مجموعة مع الإخذ بنظر الاعتبار وزن الجسم الحي، عولمت النعاج كالاتي المجموعة الأولى (سيطرة) غذيت على علفه مركزة يوميا بمعدل 350 راس، أما المجموعة الثانية أضيفت لنفس العليقة 2.5 % من بدور الكزبرة والمجموعة الثالثة أضيفت لنفس العليقة 5.0 % من بدور الكزبرة مع الرعي الحر لكل المجاميع أجريت جميع هذه المعاملات في اليوم الأول وامتدت طويلة فترة التجربة وأعطيت لقاح البروسيل في اليوم الأول بعد انتهاء تجربة التغذية. تم سحب نماذج من دم الأغنام في الأيام 7-14 - 21-28 من أيام التجربة بعد إعطاء اللقاح (لحساب النسبة المئوية لمعامل الانقسام الخيطي لخلايا البلعمة للمفيدة في الدم المحيطي وحساب النسبة المئوية لمعامل فعالية احتراق الأوكسجين بطريقة آل NBT test وحساب مستوى الأجسام المضادة ضد جرثومة البروسيل بطريقة الاليزا). أظهرت النتائج تأثير واضح لبدور نبات الكزبرة المخلوط مع العلف المستخدم في الدراسة وفي المجاميع الممنعة بلقاح البروسيل ومن خلال تحسين الاستجابة المناعية غير النوعية والمناعة الخلطية مقارنة مع مجموعة السيطرة، كما وأشارت النتائج إلى ارتفاع واضح في عياريه أضداد البروسيل بطريقة الاليزا وبفوارق إحصائية معنوية بمستوى ($P \leq 0.05$) في المجاميع الممنعة والمعاملة ببدور نبات الكزبرة بالمقارنة مع مجموعة السيطرة، وسجلت المجموعة III أعلى عياريه لمستوى الأجسام المضادة. كذلك لوحظ ارتفاع واضح عند حساب النسبة المئوية لمعامل الانقسام الخيطي لخلايا البلعمة للمفيدة في الدم المحيطي وحساب النسبة المئوية لمعامل فعالية البلعمة *phagocytic activity index* بطريقة آل NBT test وبفوارق إحصائية معنوية بمستوى ($P \leq 0.05$). من خلال النتائج المسجلة في هذه الدراسة لوحظ وجود تأثير واضح لمستخلص بدور نبات الكزبرة على مستوى الاستجابة المناعية غير الخصوصية والمناعة الخلطية لنعاج العواسية العراقية المستخدمة في هذه الدراسة.

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