Research Article

BASJVETRES

BASRAH JOURNAL OF VETERINARY RESEARCH, 2024, 23(1):1-17
https://bjvr.uobasrah.edu.iq/

Evaluation of the immunological responses elicited by the *Nigella sativa* oil and comparison with Freund's vaccine adjuvant

Maysaa A. Jumaah, Ali A. Issa Al-iedani

Department of Microbiology, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

Corresponding Author Email Address: maysaa.abdullridha@uobasrah.edu.iq
ORCID ID: https://orcid.org/0009-0006-4582-2819
DOI: https://doi.org/10.23975/bjvetr.2024.144943.1053
Received: 15 December 2023 Accepted: 8 January 2024.

Abstract

This study aimed to evaluate the adaptive and innate immune responses by preparing and evaluating the immunizing and protective efficacy of an inactivated whole Gram-negative bacteria and their crude antigens with natural adjuvant Nigella sativa (N.S) oil compared with Freund's adjuvant (FA). then detected differential and total white blood cell count in each group in addition to cytokines responses to humoral and cellular immune responses determined by ELISA. The result of this study showed that the total leukocyte count was increased in all adjuvant groups compared with a control group and the leukocyte differential count was performed and the result indicated that the lymphocyte was increased in the group of N.S. oil adjuvant, and it was statistically significant ($P < 0.0\ 1$) in N. S. adjuvant with killed bacteria (G4). In contrast, monocyte percentage elevated in the two groups of Freund's vaccine adjuvant. Concerning adaptive immune responses, Freund's vaccine adjuvant directs the immunity toward cell-mediated immunity, on the other hand, the N. S. vaccine adjuvant directs the immunity toward humoral immunity as revealed by the results of ratio of IFN-γ /IL-4. In conclusion, the uses of N. S. vaccine adjuvant directed the immunity toward TH2 responses. On the other hand, Freund's vaccine adjuvants guide the immunity toward TH1 immune responses.

Keywords: Vaccine, Adjuvant, Nigella sativa, IFN-γ, IL-4.

Introduction

A vaccine is a biological product that is the most effective means of preventing and minimizing the harm caused by infectious diseases in both humans and animals (1). It can be used to safely induce an immune response that confers protection against infection and/or disease upon subsequent exposure to a pathogen (2). Indeed, the use of adjuvants can improve this in certain vaccinations. Adjuvants are defined by Ramon as "substances used in combination with a specific antigen that produced a more robust immune response than the antigen alone" (3). The word adjuvant comes from the Latin adjuvare, which means "to help or aid." According to (4), adjuvants are thought to enhance the immune response by imitating the biological processes often linked to living infections. According to (5) Gram-negative bacteria (GNB) are responsible for a number of serious public health issues worldwide, including endotoxic shock, pneumonia, diarrhea, meningitis, urinary tract infections, and many more illnesses in humans (6). Several illnesses in ruminants, including ruminal acidosis, fatty liver syndrome, claw-related disease, retained placenta, displaced abomasum (7), and sub-acute rumen acidosis (8), are in addition to animal diseases including bovine mastitis (9).

Adjuvants are compounds that improve the capacity of vaccinations to produce immunity when administered in conjunction with vaccination antigens (10). Nonetheless, Freund's adjuvants are the most widely used emulsified adjuvants; yet, because of their excessive toxicity and reactogenicity (11), they are not permitted for use in human or veterinary medicine in the United States (12). But for a long time, not many adjuvants were added to vaccines; the most widely used adjuvants are aluminum salts (13). The drawbacks of the aluminum vaccine adjuvant include the fact that it increases the total body burden of aluminum in humans (14) and that it can, in some cases, result in granulomas and allergic reactions (15). Recent research has focused on a variety of novel compounds with effective adjuvant properties and improved safety because the toxicity and unfavorable side effects of most adjuvant formulations are the biggest issues with their use in human vaccinations, especially in routine pediatric vaccines (16).

Natural product adjuvants, such as *Nigella sativa* oil adjuvant, have historically been used extensively to help prevent and treat diseases (17). This is because these adjuvants are generally accessible, affordable, and rarely cause unfavorable side effects (18). Because of their non-specific immunostimulant effect, they could be used in place of mineral oil (19). *Nigella sativa* is a spicy, medicinal herb that is also known as black cumin or black seeds. It has been used extensively in traditional medicine and is well-known for its culinary applications. Black cumin is indigenous to much of the Indian subcontinent, the eastern Mediterranean, northern Africa, and Southwest Asia. Black cumin, a panacea, has been used in traditional medicine to treat a wide range of illnesses and ailments. (20) observed that the black seed contains p-cymene, 4-terpineol, and t-anethol in addition to other components like

carbohydrates, fats, vitamins, minerals, proteins, and essential amino acids. Moreover, nigellidine, nigellimine, nigellcine, saponine, and water-soluble triterpene are found in black seeds (21). Thymoquinone (TQ) is one of the most active ingredients and an abundant component (22). This study was aimed to evaluate the natural material's (*Nigella sativa* oil's) receptivity as an immunological adjuvant utilizing bacteria (*E. coli*) as an antigen.

Materials and methods

Laboratory animals

In all of the study's trials, thirty female Wistar albino rats weighing between 220 and 260 Grams were utilized. All of the animals were provided by Basrah University, College of Veterinary Medicine. Before being used in lab tests, rats were housed in plastic cages for two weeks in which they were allowed unlimited access to food and water. Throughout the trial, the rats were kept in controlled environments with a 12-hour light-dark cycle and a temperature between 24 and 26°C. Every animal was handled in accordance with the moral guidelines for sample collection and animal welfare.

Bacterial isolate

Local Shiga-producing *E. coli* (STEC) isolate was obtained from cattle in Basrah governorate and donated by the Department of Microbiology at the University of Basrah, College of Veterinary Medicine (23).

Preparation of killed bacteria (whole cell vaccine)

Brain-heart infusion broth was used to cultivate stock *E. Coli* at 37° C for 24 hours. While being shaken. Cells were treated with 3.7% formalin for an entire night following incubation. Following four PBS washes (24), the inactivated bacteria were adjusted to 2×10^9 CFU/ml by comparison with 0.5 McFarland Standard Solution. Until they were used, these preparations were kept at 4°C. A loopful of the dead isolates was streaked onto blood agar or MacConkey agar plates, and the plates were incubated at 37°C for 24 to 48 hours to verify sterility (25).

Preparation of crude antigens using sonication

For cell lysis, *E. coli* was suspended in phosphate buffer saline in concentration 2×10^9 CFU/ml. The sample vial was kept in an ice-water bath to prevent significant heating of the sample during sonication (26). The sample was then disrupted by sonication for 10 cycles of 60s pulse with 90s interval at a frequency of 20 kHz (27).

Preparation of *Nigella sativa* adjuvant emulsion

The emulsion was prepared by mixing the oil phase of *Nigella sativa* (N.S.) with the aqueous phase of the prepared antigen as follows:

Preparation of the oil phase of *Nigella sativa*

The oil was extracted by cold pressing of *Nigella sativa* seeds according to the method

by (28), then it was mixed with span 40 (emulsifier) (Alpha Chemicka, India) after sterilization each of them by filtration through a filter syringe 0.45µL, the mixture was mixed in a ratio of 9:1, 9 parts oil to one part of span 40 with thoroughly mixing to make an emulsion. The mixture of oil and span 40 was stored in sterile containers at room temperature until used (29).

Preparation of the Aqueous Phase of vaccine

The aqueous phase was prepared by mixing 96% inactivated *E. coli* or protein antigen solution with 4% span 40 (29).

Preparation of *E. coli* antigens and *Nigella sativa* adjuvant

Vaccines stable emulsion was prepared by thorough mixing of the prepared aqueous phase and oil phase in a ratio of 1:4, where one part of the aqueous phase was mixed with 4 parts of the oil phase with continuous mixing until production of stable emulsion (29).

Preparation of Freund's adjuvants and *E. coli* antigens

The stable water-in-oil emulsion is usually prepared by forcing the aqueous-phase antigen into an equal volume of the oil adjuvant [Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA) through three-way stopcocks cannula (12).

Vaccine dose and route of injection

In the present study, the total dose was injected subcutaneously was 0.5 ml per rat, [vaccine suspension of Freund's adjuvants vaccines or *Nigella sativa* vaccines adjuvant] (30; 31; 32).

Vaccination

Five groups (6 rats in each group) were immunized subcutaneously using standard hygiene precautions [sterile needles after having disinfected the skin of the animal with 70% ethanol] (33), each rat was injected in the dorsal region in four sites (0.1ml in three sits and 0.2ml in the fourth sit (32).

The first Group of animals was injected subcutaneously with normal saline as control. The second group (G2) was immunized with FA emulsion (industrial biotechnology, Germany), and whole killed bacteria. G3 was immunized with CFA and crude antigens of bacteria. G4 was immunized with *Nigella sativa* oil adjuvant with whole killed bacteria and G5 was immunized with *N. sativa* oil adjuvant with crude antigens of bacteria. Concerning the booster dose, all groups were injected subcutaneously using the same adjuvant vaccine 14 days later of the first injection, except the Freund's vaccine adjuvant groups which were injected with incomplete Freund's vaccine adjuvant (IFA) in the second dose.

Blood samples and plasma collection

Blood samples were collected on day fourteen after each injection, using tubes with heparin to measure the total and differential white blood cells. For plasma, collecting tubes were inverted eight times followed by centrifugation at 300 RPM in

10 min at 20°C. The tubes were stored at -20°C until analysis (34).

Total and differential leukocyte count

The total leukocyte count was performed using a Neubauer counting chamber according to (35). Whereas, the differential leukocyte count was carried out using blood smears stained with Giemsa stain according to (36).

Measurement of the concentration of IL-4 and IFN-γ

The concentration of cytokines (IL-4 and IFN-γ) was measured in the plasma of rats using ELISA kits purchased from (Bioassay Technology Laboratory/ China), these kits utilized the quantitative sandwich enzyme immunoassay according to the manufacturer's protocol. Cytokine protein quantification was determined by comparing samples to the standard curve generated from the respective kits.

Statistical analysis

The statistical analysis of results to determine whether differences exist among the means of five groups was performed using GraphPad Prism version 8.

Results

Changes in total and differential leukocyte counts according to type of used vaccine adjuvant

Concerning total leukocyte count the count was increased in all adjuvant groups compared with a control group and these increases were statistically significant in (G1, G3, G4) groups, Table (1), and (Figure (1). The leukocyte differential count was performed for five main types of leukocytes, i.e., neutrophils, eosinophils, basophils, monocytes, and lymphocytes, and variably increased in WBC differentials, (Table 1, and Figure 2).

The analysis of hematological data revealed that all groups have no increase in neutrophil percentage. The lymphocyte was increased in the group of *N. sativa* oil adjuvant and was statistically significant in (G4). Monocyte percentage revealed increase in two groups in Freund's vaccine adjuvant. While, all groups have no significant increase in eosinophil and no change in basophil (zero value).

Table 1. Hematological parameters (WBC and differential count), induced by two types of vaccine adjuvants compared with the negative control group

Group	Neutrophils%	Lymphocytes%	Eosinophils %	Basophils %	Monocytes%	white blood cell count
G1	21.875∓ 1.209	68.333∓2. 211	0.865∓ 0.7544	0	8.9275∓04. 728	6.805×10^{3} ∓0.7068
G2	20.593∓ 2.638 ns	57.508∓1. 897 ****	1.305∓0.814 1 ns	0	20.593∓3. 857 ****	9.7×10^{3}∓0. 33314 ****
G3	19.68∓3. 047 *	60.455∓3.53 6 ****	0.8375∓0.97 25 ns	0	19.027∓5. 710 ****	9.2×10^{3}∓0.3955 ****
G4	15.73∓1.74 8 *	75.425∓2. 048 **	0.5775∓ 0.3983 ns	0	8.2625∓0. 607 ns	7.656 $\times10^{3}$∓0.3620 **
G5	17.53∓1. 604 ns	72.892∓1.49 7 ns	0.695 ∓0.440 ns	0	9.13∓0.7824 ns	7.363×10^{3}∓ 0.2264 ns

-All data presented as mean ± standard deviation of the differential and total white blood cells count

**** P < 0.0001, *** P < 0.001, ** P < 0.0 1, * P < 0.05, ns not significant

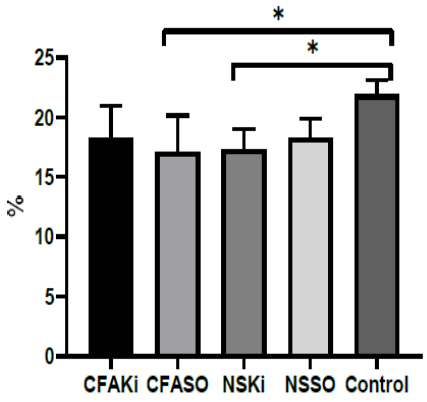
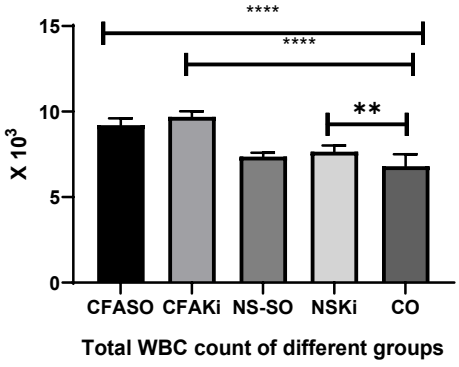
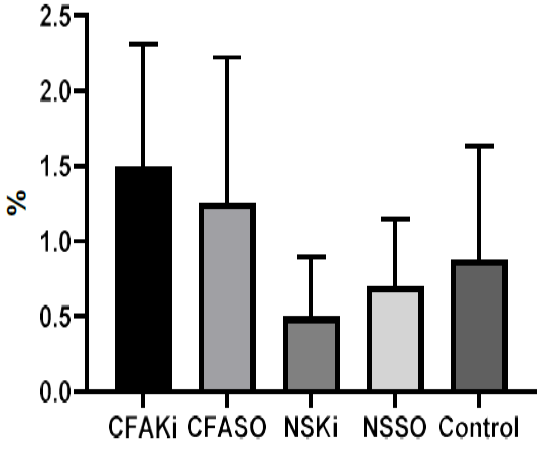


Figure (1): Total white blood cells count in different groups

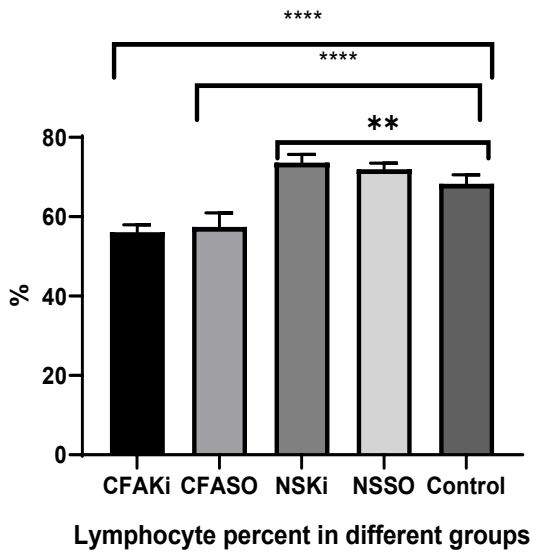
Note: G1=CO, G2=CFAKi, G3=CFASO, G4=NSKi, G5=NS-S0



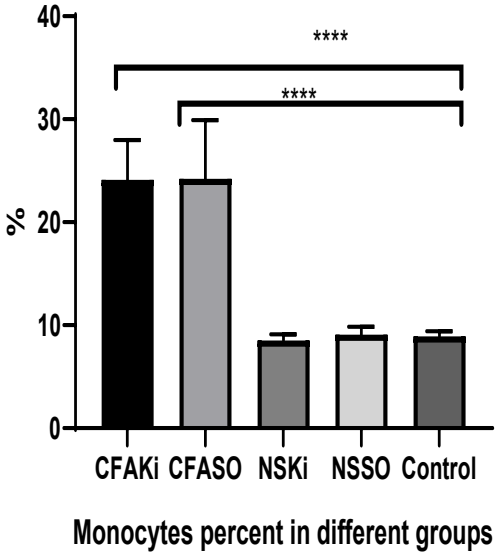
A- Neutrophil percent in different groups



B- Eosinophil percent in different groups



C- lymphocyte percent in different groups



D-monocyte percent in different groups

Figure (2): Effects of different adjuvants on the differential white blood cell count

Cytokine measurement

The concentration of IFN-γ and IL-4 in the two types of vaccine adjuvant after the second subcutaneous doses is shown in Table (2). In addition, the ratio of IFN-γ to IL-4 was determined as a secondary indicator for the type of response, according to (37, 38) who noted that the response was

considered as a Th1 if the IFN-γ /IL-4 ratio exceeded one. However, a Th2 response if the ratio was less than one. The ratio of IFN-γ to IL-4 indicated the humoral responses in (G1, G2, G4, G5), however, the ratio of G3 represents cellular responses, (Table 2).

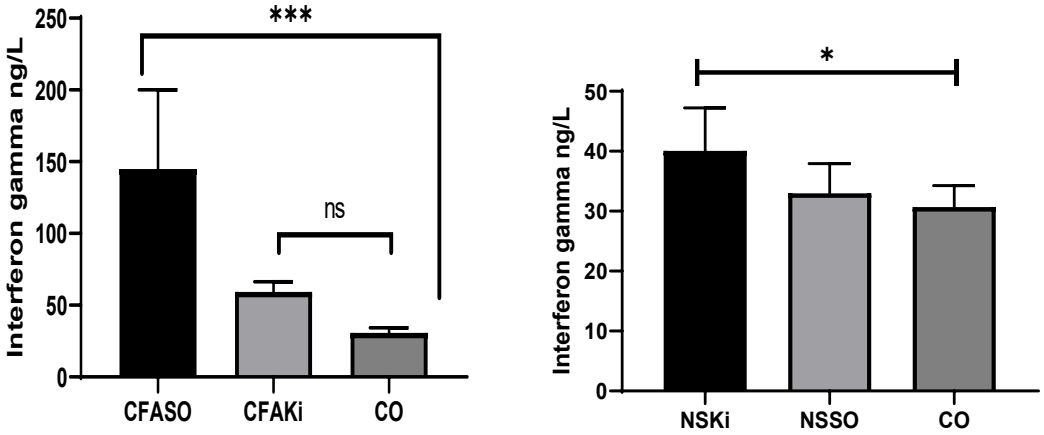
The concentration of IL-4 in G4 and G5 was statistically significant (Figure 4). Regarding the IFN-γ the increase was statistically significant in G3 and G4, (Figure 3).

Table 2. The concentration of IFN-γ and IL-4 of different vaccine adjuvants and the ratio of the two interleukins compared with control

Groups	IL-4	IFN-γ	IFN-γ /IL-4
G1	32.105∓4.77215	30.66∓3.6085	0.967∓0.137
G2	170.04 ∓119.56 ns	59.28∓7.08929 ns	0.5015∓0.4853
G3	130.133 ∓ 108.25 ns	144.975∓ 55.1537 ***	3.5807∓3.448
G4	62.09∓11.0305 ****	40.07∓7.14681 *	0.6586∓0.1464
G5	49.3013∓4.79651 **	32.9838∓4.95629 ns	0.674∓0.1209

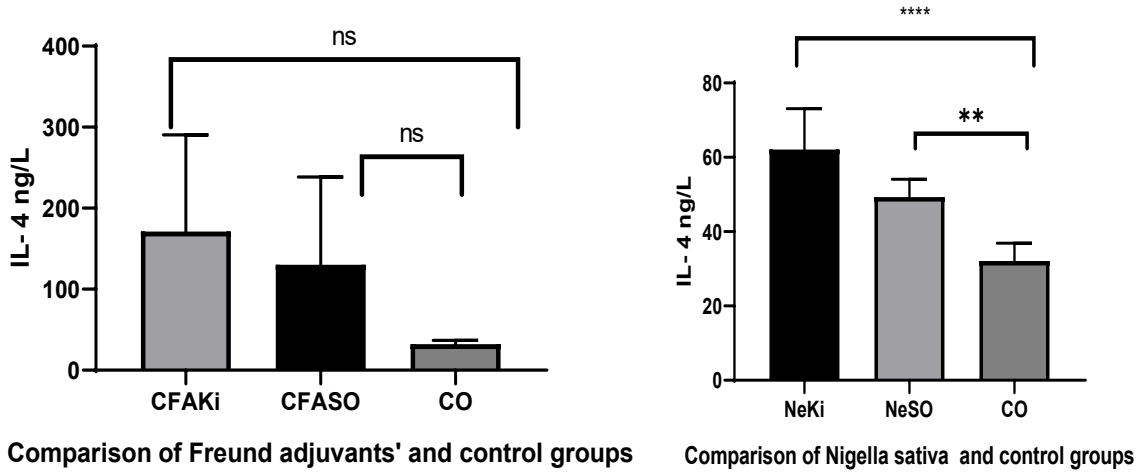
All data presented as mean ± standard deviation of the interleukins

**** P < 0.0001, *** P < 0.001, ** P < 0.0 1, * P < 0.05, ns not significant



A-Comparison of Freund adjuvants and control groups **B- Comparison of *Nigella sativa* and control groups**

Figure (4): Concentration of IFN-γ in the two vaccine adjuvants in rats



A- Comparison of Freund vaccine adjuvant and control vaccine adjuvant and control. **B- Comparison *of Nigella sativa* oil**

Figure (5): Concentration of IL-4 in the two vaccine adjuvants in rats

Discussion

The effects of medicinal plants on various animals' immune systems are well-established and verified (39). These are impacted by non-specific immune activation that affects the host's humoral or cellular immune system (40).

Medicinal plants that have a good effect and no side effects have been frequently recommended (41). One such plant is *Nigella sativa*, which has a nonspecific immunostimulant effect. Several studies have demonstrated that *Nigella sativa* oil can stimulate humoral and cellular immune responses (29).

In the current investigation, Gram-negative bacteria antigen was added to *N. sativa* essential oils as an adjuvant. The findings were evaluated two weeks after two vaccination doses, and the results were compared with the gold standard, Freund's vaccine adjuvant (FA).

According to (42), the innate immune response is the first line of defense for the host. Adjuvants may trigger innate immune responses at the injection site. Adjuvants may alter the type and amount of adaptive immune responses, such as leukocytes, which are white blood cells that are essential to the body's defensive mechanism, based on the innate reactions that have been triggered (43). When there is inflammation, risks are removed from the site of infection or damage by inflammatory cells such as neutrophils and monocytes, other leukocytes, and plasma components (44).

Similar to the findings reported by (45), the results demonstrated a considerable rise in the total WBC count following the injection of Freund's vaccine adjuvant (FA) in two groups compared with the normal control group. Overall white blood cell counts increased significantly, particularly monocyte counts, which increased statistically significantly in the two FA groups. (46) earlier reported similar results, noting that the presence of Mycobacterium spp. antigens in CFA elveated this relative to control groups (47). They also noticed a higher ratio of monocytes to lymphocytes. Comparing the eosinophil findings with the control group, the adjuvant effect of the FA vaccination was not statistically significant. However, the basophil outcome was zero for all groups. While, checking the stained smear, these results are comparable to that recorded by (48).

Concerning the total WBC count of *N. sativa* groups, the increase is significant in the group of *N. sativa* with whole killed bacteria and a non-significant increase with sonicated bacterial antigen compared with normal control, similar results were recorded by (40). Moreover, there was an increase in total white blood cells, especially lymphocytes in the two groups of *Nigella sativa* oil adjuvant. There was a significant increase with whole bacteria antigen and no significant with sonicated antigen. The two groups have no significant changes in monocyte counts, the result of eosinophil and basophil similar to that reported by (49).

In response to damaging stimuli, endogenous mediators carry out defensive biological processes known as inflammatory

responses. (50) state that cytokines are among the most prevalent inflammatory mediators. They are often released by neutrophils and macrophages as well as by the wounded tissue itself.

After being activated, CD4+ T helper cells proceed on to develop into Th1 or Th2 cells, which secrete Th1 cytokines (IL-2, IL-12, IFN-γ, and TNFα) and Th2 cytokines (IL-4, IL-5, IL-10, and IL-13 with specialization). Since the choice to differentiate into Th1 or Th2 cells ultimately tips the scales towards the direction of a humoral or cellular immune response, agents that can affect the Th1/Th2 balance may be able to change how the adaptive immune response plays out in a variety of illnesses and medical conditions (51).

According to the results, the adjuvant from the *N. sativa* oil promoted the production of Th2 cytokines more than Th1 cytokines. This finding is consistent with that published by (49) and is comparable to that of (51).

Cell-mediated immunity is primarily stimulated by the mycobacterial component of CFA, although antibody responses can be significantly increased by emulsifying antigens in paraffin oil or surfactant alone IFA (52). Furthermore, (53) proposed that IFA vaccination is an efficient way to stimulate T-cell and antibody responses. On the other hand, opinions about how well vaccination with peptides in IFA induces T-cell responses have diverged. Indeed, in certain cases, a second dose of IFA vaccination may result in a decrease in cell-mediated responses (54).

It is challenging to explain how the kind of immunization agent affects the effect of the vaccine adjuvant on immune responses differently. The difference between soluble protein and suspended intact bacteria in the antigen may be the reason for this. Furthermore, there might be a function for chemical composition (55).

According to this research, the type of antigen may influence how the body reacts to the vaccine, explaining why the two types of CFA have different immune responses: CFA containing whole bacteria induces humoral immunity. While, CFA containing sonicated bacterial antigens induces cellular immunity because free LPS stimulates TLR4, which in turn causes TH1 (56), and CFA containing killed bacteria induces humoral immunity (57).

While, Freund's adjuvant stimulates cellular immune responses, the *N. sativa* oil vaccination adjuvant stimulates humoral adaptive immunity when combined with particulate antigen (dead bacteria) or soluble sonicated bacteria.

Conclusion: Freund's adjuvant directs the immunity toward cell-mediated immunity. On the other hand, the N. S. adjuvant directs the immunity toward humoral immunity. This research is considered one of the important studies in the field of immunity using black seed oil (N.S.) as a natural immune aid to induce adaptive and innate immune responses and comparing it with the manufactured vaccine, the use of the ELISA test to detect innate and cellular immune responses against foreign bodies is one of the good tests for detecting concentrations of

cytokines and interleukins. Another test can be used for more advanced studies using gene expression, through the use of Real-time PCR.

Conflict of Interest: The authors declare no conflict of interest.

Funding statement: Self-funding.

Reference

1.Nooraei, S., Lotfabadi, A. S., Akbarzadehmoallemkolaei, M., and Rezaei, N. (2023). Immunogenicity of Different Types of Adjuvants and Nano-Adjuvants in Veterinary Vaccines: A Comprehensive Review. *Vaccines, 11*(2): 453-472. https://doi.org/10.3390/vaccines11020453

2. Pollard, A. J. and Bijker, E. M. (2021). A guide to vaccinology: from basic principles to new developments. *Nat Rev Immunol 21*: 83–100. https://doi.org/10.1038/s41577-020-00479-7.

3.Awate, S., Babiuk, L. A., and Mutwiri, G. (2013). Mechanisms of action of adjuvants. *Frontiers in immunology, 4*: 114 – 123. https://doi.org/10.3389/fimmu.2013.00114.

4. De Gregorio, E., Tritto, E. and Rappuoli, R. (2008). Alum adjuvanticity: unraveling a century-old mystery. *Eur. J. Immunol. 38*, 2068–2071. DOI: 10.4172/1948-5964.1000155.

5. Oliveira, J. and Reygaert, W. C. (2023). Gram-Negative Bacteria. [Updated 2022 Oct 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK538213/

6. Ng, L. S., Tan, T. Y., and Yeow, S. C. (2010). A cost-effective method for the presumptive identification of Enterobacteriaceae for diagnostic microbiology laboratories. *Pathology, 42*(3), 280–283. https://doi.org/10.3109/00313021003631338

7.Reisinger, N., Wendner, D., Schauerhuber, N., and Mayer, E. (2021). Effect of Lipopolysaccharides (LPS) and Lipoteichoic Acid (LTA) on the Inflammatory Response in Rumen Epithelial Cells (REC) and the Impact of LPS on Claw Explants. *Animals: an open access journal from MDPI, 11*(7): 2058 – 2076. https://doi.org/10.3390/ani11072058

8. Eckel, E. F. and Ametaj, B. N. (2016). Invited review: Role of bacterial endotoxins in the etiopathogenesis of periparturient diseases of transition dairy cows. *J. Dairy Sci. 99*:5967–5990. doi: 10.3168/jds.2015-10727.

9.Cheng, W. N. and Han, S. G. (2020). Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments - A review. *Asian-Australasian journal of animal sciences, 33*(11), 1699–1713. https://doi.org/10.5713/ajas.20.0156.

10.Pulendran, B., Arunachalam, S. P. and O'Hagan, D. T. (2021). Emerging concepts in the science of vaccine adjuvants. *Nat Rev Drug Discov 20*, 454–475, https://doi.org/10.1038/s41573- 021-00163-y.

11.Zhang, J., Miao, J., Han, X. lu, Y., Deng, B., Lv, F, Zhao, Y., Ding, C., and Hou, J. (2018). Development of a novel oil-in-water emulsion and evaluation of its potential adjuvant function in a swine influenza vaccine in mice. *BMC Vet Res 14*: 415 – 425. https://doi.org/10.1186/s12917-018-1719-2.

12.Stills, H. F and Jr (2005). Adjuvants and antibody production: dispelling the myths associated with Freund's complete and other

adjuvants. *ILAR journal, 46*(3), 280–293. https://doi.org/10.1093/ilar.46.3.280.

13. Facciolà, A., Visalli, G., Laganà, A., and Di Pietro, A. (2022). An Overview of Vaccine Adjuvants: Current Evidence and Future Perspectives. *Vaccines, 10*(5): 819 - 844. https://doi.org/10.3390/vaccines10050819.

14.Kramer, M. F. and Heath, M. D. (2014). Aluminium in allergen-specific subcutaneous immunotherapy--*a German perspective. Vaccine, 32*(33), 4140–4148. https://doi.org/10.1016/j.vaccine.2014.05.063

15.Nicholls, E. F., Madera, L., and Hancock, R. E. (2010). Immunomodulators as adjuvants for vaccines and antimicrobial therapy. *Annals of the New York Academy of Sciences, 1213*, 46–61. https://doi.org/10.1111/j.1749-6632.2010.05787.

16. Gupta, R. K., and Siber, G. R. (1995). Adjuvants for human vaccines--current status, problems and future prospects. *Vaccine, 13*(14), 1263–1276. https://doi.org/10.1016/0264-410x(95)00011-o

17.Saklani, A. and Kutty, S. K. (2008). Plant-derived compounds in clinical trials. *Drug Discov Today 13* (3-4) .161–171, https://doi.org/10.1016/j.drudis.2007.10.010.

18.Berretta, A. A., Silveira, M. A. D., Capcha, C. J. M., and De Jong, D. (2020). Propolis and its potential against SARS-CoV-2 infection mechanisms and COVID-19 disease: Running title: Propolis against SARS-CoV-2 infection and COVID-19. *Biomedicine and pharmacotherapy = Biomedicine and pharmacotherapy, 131*: 110622 - 110638. https://doi.org/10.1016/j.biopha.2020.110622.

19.Hussien, A. S. and Madbouly, H. M. (2005). Vaccination of chickens with live Newcastle disease virus vaccines adjuvant with Nigella sativa oil. *Journal of Veterinary Medical Research. No. 2*, (15), 123-126.

20.Hannan, M. A., Rahman, M. A., Sohag, A. A. M., Uddin, M. J., Dash, R., Sikder, M. H., Rahman, M. S., Timalsina, B., Munni, Y. A., Sarker, P. P., Alam, M., Mohibbullah, M., Haque, M. N., Jahan, I., Hossain, M. T., Afrin, T., Rahman, M. M., Tahjib-Ul-Arif, M., Mitra, S., Oktaviani, D. F., … Kim, B. (2021). Black Cumin (Nigella sativa L.): A Comprehensive Review on Phytochemistry, Health Benefits, Molecular Pharmacology, and Safety. *Nutrients, 13*(6): 1784 - 1843. https://doi.org/10.3390/nu13061784

21.Rahmani, A. H., Alzohairy, M. A., Khan, M. A., and Aly, S. M. (2014). Therapeutic Implications of Black Seed and Its Constituent Thymoquinone in the Prevention of Cancer through Inactivation and Activation of Molecular Pathways. *Evidence-based complementary and alternative medicine: eCAM, 2014*(3):724658 - 724670. https://doi.org/10.1155/2014/724658.

22.Forouzanfar, F., Bazzaz, B. S. and Hosseinzadeh, H. (2014). Black cumin (Nigella sativa) and its constituent (thymoquinone): a review on antimicrobial effects. *Iranian journal of basic medical sciences, 17*(12), 929–938.

23.Farhan, Z. and AL-Iedani. (2019). A. Molecular detection of shiga toxin (stx1 and stx2) and intimin (eae A) genes in Escherichia coli isolated from fecal samples of cattle, sheep, and human in Basrah governorate. *Basrah J Vet Res.18*(2):288-305.

24. Sunwoo, H. H., Wang, W. W., and Sim, J. S. (2006). Detection of Escherichia coli O157:H7 using chicken immunoglobulin Y. *Immunology letters, 106*(2), 191–193. https://doi.org/10.1016/j.imlet.2006.05.005.

25.Manzoor, S., Rahman, S., Ashraf, M., Khan, F. M., Hussain, Z. and Ali, S. A. (2017). Determination of Adsorption Capacity of Alum Hydroxide {Al (OH) 3} Gel for Streptococcus equi sub specie equi and Streptococcus dysgalacltae sub species equisimillis. *J Antivir Antiretrovir 9*(1): 006-009. doi:10.4172/1948-5964.1000155

26. Shrestha, P., Holland, T. M., and Bundy, B. C. (2012). Streamlined extract preparation for Escherichia coli-based cell-free protein synthesis by sonication or bead vortex mixing. *BioTechniques, 53*(3), 163–174. https://doi.org/10.2144/0000113924.

27. Shrestha, P., Holland, T. M., and Bundy, B. C. (2012). Streamlined extract preparation for Escherichia coli-based cell-free protein synthesis by sonication or bead vortex mixing. *BioTechniques, 53*(3), 163–174. https://doi.org/10.2144/0000113924

28. Kiralan M., Özkan G., Bayrak A. and Ramadan M.F. (2014). Physicochemical properties and stability of black cumin (Nigella sativa) seed oil as affected by different extraction methods. *Industrial Crops Prod. 57*:52–58. doi: 10.1016/j.indcrop.2014.03.026.

29. Mady, W. H., Arafa, A., Hussein, A. S., Aly, M. M. and Madbouly, H. M. (2013). Nigella Sativa Oil as an Immunostimulant Adjuvant in H5 Based DNA Vaccine of H5N1 Avian Influenza Virus. *Global Veterinaria 10* (6): 663-668.

30. Katherine, E. Hanlon, and Vanderah, T. W. (2010). Chapter One – Constitutive Activity at the Cannabinoid CB1 Receptor and Behavioral Responses, Editor(s): P. Michael Conn, *Methods in Enzymology, Volume 484*, Pages 3-30,ISSN 0076-6879, ISBN 9780123812988, https://doi.org/10.1016/B978-0-12-381298-8.00001-0.

31. Razin, M. A. F., Osman, A., Ali, M. A., Bahgat, M. M., and Maghraby, A. S. (2017). Immune responses to killed reassorted influenza virus supplemented with natural adjuvants. *Acta microbiologica et immunologica Hungarica, 64(*3), 313–330. https://doi.org/10.1556/030.64.2017.011

32. IACUCs [Institutional Animal Care and Use Committee] (2022). Administering Complete Freund's Adjuvant (CFA) and other Adjuvants.

33.Lindblad, E. B. (2008). Freund's Adjuvants, DOI: 10.1385/1-59259-083-7:49, In book: Vaccine Adjuvants (pp.49-63).

34. Sotelo-Orozco, J., Chen, S. Y., Hertz-Picciotto, I., and Slupsky, C. M. (2021). A Comparison of Serum and Plasma Blood Collection Tubes for the Integration of Epidemiological and Metabolomics Data. *Frontiers in molecular biosciences, 8*: 682134 - 682150. https://doi.org/10.3389/fmolb.2021.682134

35. Nurhayati, A. P. D., Pratiwi, R. and Soekardiman, S. (2017). Total and Differential Cells of Leukocyte Mice (Mus musculus) on Evaluation in Vivo Anticancer Extracts Ethanol Marine Sponges Aaptos suberitoides. *J. Phys. Conf. Ser., 1374*: 012031- 012036.

36. Sulistyaningsih, E., Wibisono, R., and Dewi, R. (2022). Leukocyte and IgM Responses to Immunization with the CIDR1α-PfEMP1 Recombinant Protein in the Wistar Rat. *Tropical medicine and infectious disease, 7*(9): 222 - 232. https://doi.org/10.3390/tropicalmed7090222

37. Mozaffari, H. R., Molavi, M., Lopez-Jornet, P., Sadeghi, M., Safaei, M., Imani, M. M., Sharifi, R., Moradpoor, H., Golshah, A., and Jamshidy, L. (2019). Salivary and Serum Interferon-Gamma/Interleukin-4 Ratio in Oral Lichen Planus Patients: A Systematic Review and Meta-Analysis. *Medicina (Kaunas,*

Lithuania), *55*(6): 257 - 266. https://doi.org/10.3390/medicina55060257

38. Jensen, F. C., Savary, J. R., Diveley, J. P., and Chang, J. C. (1998). Adjuvant activity of incomplete Freund's adjuvant. *Advanced drug delivery reviews,* *32*(3), 173–186. https://doi.org/10.1016/s0169-409x(98)00009-x.

39. Alhazmi, H. A., Najmi, A., Javed, S. A., Sultana, S., Al Bratty, M., Makeen, H. A., Meraya, A. M., Ahsan, W., Mohan, S., Taha, M. M. E., and Khalid, A. (2021). Medicinal Plants and Isolated Molecules Demonstrating Immunomodulation Activity as Potential Alternative Therapies for Viral Diseases Including COVID-19. *Frontiers in immunology, 12* : 637553 - 637576. https://doi.org/10.3389/fimmu.2021.637553.

40. Raissy M., Hashemi S., Roushan M., Jaafarian M., Momtaz H., Soltani M. and Kheirabad, P. E. (2018). Effects of essential oils of Satureja bachtiarica and Nigella sativa on the efficacy of lactococcosis vaccine in rainbow trout (Oncorhynchus mykiss) *Iranian Journal of Fisheries Sciences* *17*(1) 95-106 DOI: 10.22092/IJFS.2018.115587.

41.Alishahi, M. and Mesbah, M. (2012). Effects of Viscum album and Nigella 27 sativa extracts on survival rate, growth factors and resistance to Aeromonas hydrophila infection in gold fish (Carassius auratus). *Journal of Veterinary Researches, 67*(3), 285-290.

42.Mogensen T. H. (2009). Pathogen recognition and inflammatory signaling in innate immune defenses. *Clinical microbiology reviews,* *22*(2), 240–273. https://doi.org/10.1128/CMR.00046-08

43. Bolhassani, A., Talebi, S., and Anvar, A. (2017). Endogenous and Exogenous Natural Adjuvants for Vaccine Development. *Mini reviews in medicinal chemistry, 17(15)*,1442–1456. https://doi.org/10.2174/1389557517666170228115801.

44. Shaaban, H. H., Hozayen, W. G., Khaliefa, A. K., El-Kenawy, A. E., Ali, T. M., and Ahmed, O. M. (2022). Diosmin and Trolox Have Anti-Arthritic, Anti-Inflammatory and Antioxidant Potencies in Complete Freund's Adjuvant-Induced Arthritic Male Wistar Rats: Roles of NF-κB, iNOS, Nrf2 and MMPs. *Antioxidants (Basel, Switzerland), 11*(9): 1721 - 1740. https://doi.org/10.3390/antiox11091721.

45. Zhang, Z., Wang, X., Li, P., Bai, M. and Qi, M. (2020). Evaluation of antiarthritic activity of ginkgolic acid against Freund's adjuvant induced arthritic rat model. *Brazilian Journal of Pharmaceutical Sciences;56*: 18638- 18647.

46. Mahdi, H. J., Khan, N. A. K., Asmawi, M. Z. B., Mahmud, R., and Murugaiyah, V. A/L. (2018). In vivo anti-arthritic and anti-nociceptive effects of ethanol extract of Moringa oleifera leaves on complete Freund's adjuvant (CFA)-induced arthritis in rats. *Integrative medicine research,* *7*(1), 85–94. https://doi.org/10.1016/j.imr.2017.11.002.

47. Iqbal, S., Ahmed, U., and Zaidi, S. B. H. (2014). Monocyte lymphocyte ratio as a possible prognostic marker in antituberculosis therapy. *Journal of Rawalpindi Medical College (JRMC);* *18*(2):178-181 https://www.journalrmc.com/index.php/JRMC/article/view/330

48. Noh, A. S. M., Chuan, T. D., Khir, N. A. M., Zin, A. A. M., Ghazali, A. K., Long, I., A. b Aziz, C. B., and Ismail, C. A. N. (2021). Effects of different doses of complete Freund's adjuvant on nociceptive behaviour and inflammatory parameters in polyarthritic rat model mimicking rheumatoid arthritis. *PloS one, 16*(12), e0260423

-0260446. https://doi.org/10.1371/journal.pone.0260423.

49.Hailat, N., Al-Kahil S., Alkofahi, A., Lafi, S., Al-Ani. F., Al-Darraji, A. and Bataineh, Z. (1998). Effects of Nigella sativa Extracts on Antibody Response of Rats Vaccinated with Brucella Vaccine (Rev-1), *Pharmaceutical Biology, 36*:3, 217-221, DOI: 10.1076/phbi.36.3.217.6338.

50. Majdalawieh, A., and Ro, H. S. (2010). Regulation of IκBα function and NF-κB signaling: AEBP1 is a novel proinflammatory mediator in macrophages. *Mediators of inflammation: 823821- 823847*. https://doi.org/10.1155/2010/823821.

51. Majdalawieh, A. F. and Fayyad, M. W. (2015). Immunomodulatory and anti-inflammatory action of Nigella sativa and thymoquinone: A comprehensive review, *International Immunopharmacology, 28*, Issue 1, Pages 295-304, ISSN 1567-5769, https://doi.org/10.1016/j.intimp.2015.06.023.52. Coffman, R. L., Sher, A., and Seder, R. A. (2010). Vaccine adjuvants: putting innate *immunity to work. Immunity, 33*(4), 492–503. https://doi.org/10.1016/j.immuni.2010.10.002.

53. Melssen, M. M., Fisher, C. T., Slingluff, C. L. and et al .(2022). Peptide emulsions in incomplete Freund's adjuvant create effective nurseries promoting egress of systemic CD4+ and CD8+ T cells for immunotherapy of cancer. *Journal for Immuno Therapy of Cancer ;10*: e004709 - 4723. doi: 10.1136/jitc-004709.

54.Hossain, A., Zheng, C. L., Kukita, A., and Kohashi, O. (2001). Balance of Th1/Th2 cytokines associated with the preventive effect of incomplete Freund's adjuvant on the development of adjuvant arthritis in LEW rats. *Journal of autoimmunity, 17*(4), 289–295.

55. Soliman, R. T., Salama, S. S., Sayed, R. H., Galal, H. M. and Tawfek, A. M. (2015). Preparation of Combined Inactivated Vaccine Against Salmonella enteritidis, Salmonella typhimurium and Clostridium perfringens type A and C Toxins, *World Applied Sciences Journal 33* (3): 472-478, 2015, ISSN 1818-4952, DOI: 10.5829/idosi.wasj.2015.33.03.411.

56. Mukherjee, S., Chen, L. Y., Papadimos, T. J., Huang, S., Zuraw, B. L., and Pan, Z. K. (2009). Lipopolysaccharide-driven Th2 cytokine production in macrophages is regulated by both MyD88 and TRAM. *The Journal of biological chemistry, 284*(43), 29391–29398. https://doi.org/10.1074/jbc.M109.005272

57.Osterloh, A. (2022). Vaccination against Bacterial Infections: Challenges, Progress, and New Approaches with a Focus on Intracellular Bacteria. *Vaccines. 10*(5):751- 783.

تقييم الاستجابات المناعية التي أثارها زيت حبة البركة ومقارنتها مع مساعد اللقاح لفرويند

ميساء عبد الرضا جمعة وعلي عبود عيسى العيداني

فرع الأحياء الدقيقة، كلية الطب البيطري، جامعة البصرة، البصرة، العراق

الخلاصة

صممت هذه الدراسة لتقييم الاستجابات المناعية التكيفية والفطرية من خلال تحضير وتقييم الفعالية التحصينية والوقائية للبكتيريا سالبة الجرام المعطلة ومستضداتها الخام باستخدام زيت حبة البركة (N.S.) كمساعد مناعي طبيعي ومقارنته مع مساعد فرويند (FA) ثم تقدير اعداد خلايا الدم البيضاء التفاضلي والإجمالي لكل مجموعة بالإضافة إلى استجابات السيتوكينات الخاصة بالاستجابات المناعية الخلطية والخلوية التي تم تحديدها باستخدام عدة اليزا. أظهرت نتائج هذه الدراسة أن العدد الكلي للكريات البيض ارتفع في جميع المجموعات التي اعطيت المساعدات المناعية مقارنة مع مجموعة السيطرة وتم إجراء العد التفريقي للكريات البيض وأشارت النتيجة إلى زيادة عدد الكريات الليمفاوية في مجموعة المادة المساعدة للزيت N.S. وكانت ذات دلالة إحصائية في مجموعة المادة المساعدة لزيت N.S. مع البكتيريا الميتة (4G) في المقابل، ارتفعت نسبة خلايا الوحيدة في مجموعتي لقاح فرويند المساعد. فيما يتعلق بالاستجابات المناعية التكيفية، فإن مساعد لقاح فرويند وجه المناعة نحو المناعة الخلوية، ومن ناحية أخرى فإن مساعد لقاح N. S. وجه المناعة نحو المناعة الخلطية كما أظهرت نتائج نسبة IL/ γ-IFN-4. نستنتج من هذه الدراسة فإن استخدامات اللقاح المساعد N. S. وجهت المناعة نحو استجابات TH2، من ناحية أخرى، فإن مساعد اللقاح لفرويند وجه المناعة نحو الاستجابات المناعية TH1.

الكلمات المفتاحية: لقاح، مساعد، حبة البركة، γ-IFN ،I-4