

# Cytokine Profiles of the Pro-Inflammatory and Anti-Inflammatory Response to Bacterial Antigens when Combined with Vaccine Adjuvant

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**Abstract** | This study aim to investiage a natural alternative (alcoholic extract of propolis and nigella oil) to current vaccine adjuvants (alum) using inactivated antigens (sonicated and whole-killed bacteria) of *E. coli*. We also aimed to reveal tthe nature of host innate and adaptive immune responses by measuring the extent of their effects on the level of pro-inflammatory (interferon  $\gamma$  and interleukins (IL)-12) and anti-inflammatory cytokines (IL-4 and IL-10) and innate immune responses (total white blood count, phagocytosis and respiratory burst). Our results demonstrated that all vaccines have induced both innate and adaptive immune responses. However, the adjuvant with alum hydroxide mixed with sonicated antigens and killed bacteria induced higher pro-inflammatory cytokine effects (IFN-  $\gamma$   $P < 0.05$ ,  $P < 0.001$ ) and anti-inflammatory cytokines (IL-4  $P < 0.0001$ ,  $P < 0.001$ ), respectively. Interestingly, all immunized groups switched immune responses toward Th2 and reduced pro-inflammatory cytokines to produce homeostasis and reduce damage to body tissue. In conclusion, the use of natural vaccine adjuvants and alum led to a type 2 immune response and diverted immunity towards Th2. Importantly, all adjuvant types increased the level of innate immune responses. However, alum performed better in induction of adaptive immunity than the rest of adjuvants used.

**Keywords** | Alcoholic extract of propolis, *Nigella sativa*, Interferon, Interleukin, Iraq

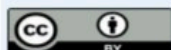
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## INTRODUCTION

Vaccines are significant medical achievements, preventing diseases by inducing the immune response. Purified antigen-based vaccines require adjuvants to improve their strength, quality, and duration (Martión et al., 2019). Adjuvants are substances that are injected with an antigen to boost the antigen-stimulating humoral and/or cell-mediated immune response. Adjuvants can modify the immune system reaction to the antigen and often allow for the use of lower antigen doses. There have been almost a hundred adjuvant preparations described in previous years

(Vogel and Powell, 1995). Aluminum-based adjuvant will remain a crucial part of current and upcoming approved vaccines, particularly significant combination vaccines (Laera et al., 2023). However, it has side effects that increase the risk of autoimmunity, chronic brain inflammation, and related neurological problems; These can have serious and far-reaching negative effects on health (Tomljenovic and Shaw, 2011), so it is necessary to find a new adjuvant that can replace the alum-type adjuvant (Sivakumar et al., 2011), so drug research now focuses more on natural medicine than on synthetic drugs (Fan et al., 2015).