# Immunomodulatory Effects of Perorally Administered Propolis in Autoimmune Diabetes: A Preclinical Study in NOD Mice

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## Abstract

Propolis, a polyphenol-rich resin produced by honey bees, exhibits both anti-inflammatory and immunostimulatory effects through modulation of NF-κB and Toll-like receptor 4 (TLR4) pathways. Given this dual activity, propolis may hold therapeutic relevance in autoimmune diseases such as type 1 diabetes (T1D). The Non-Obese Diabetic (NOD) mouse provides a well-established model for investigating immunomodulatory interventions in T1D.

Female NOD mice (n = 66) were randomized to receive control chow or chow supplemented with 1.8% (low dose) or 3.6% (high dose) raw propolis from 4 weeks of age. Diabetes incidence was monitored until 30 weeks. At 14 weeks, pancreatic insulitis was assessed histologically, T cell subsets in pancreatic lymph nodes and spleen were analyzed by flow cytometry, serum cytokines were measured using multiplex assays, and gut microbiota composition was profiled by full-length 16S rRNA sequencing in 6 mice from each group.

Propolis delayed diabetes onset, with significance at 17 weeks, although cumulative incidence converged by 30 weeks. High dose propolis reduced islet immune infiltration and significantly lowered $CD4^+$ T helper cell counts in pancreatic lymph nodes, while increasing T cell counts in the spleen. Cytokine analysis revealed reduced IL-4, elevated IL-10, and increased IFN-γ in the high dose group, indicating a Th1-skewed yet regulated immune profile. Classical pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) were unaffected. Gut microbiota analysis showed distinct compositional changes, including enrichment of *Akkermansia muciniphila* and *Heminiphilus faecis* in the high dose group.

Thus, peroral propolis modulates systemic and local immune responses in NOD mice, delays diabetes onset, and alters gut microbiota, supporting its potential as a dietary adjunct in autoimmune diabetes prevention.