



## Full-Length Article

# Holistic modulation of TLR4, MAPK, and apoptosis Signalings and gut microbiota by Sihuangzhili granule: A herbal strategy against avian colibacillosis

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## ABSTRACT

Avian pathogenic *Escherichia coli* (APEC) poses a significant threat to the poultry industry due to antibiotic resistance. This study evaluated the therapeutic efficacy of the Chinese herbal formula Sihuangzhili Granule (SHZL) against avian colibacillosis in comparison to treatment with oxytetracycline. Physiological, histopathological, and biochemical analyses revealed that SHZL alleviated APEC-induced diarrhea, restored organ condition (heart, liver, and intestine), and normalized serum metabolic markers more effectively than oxytetracycline. Bioinformatic analysis identified 59 shared targets between SHZL and the disease phenotypes of pericarditis, hepatitis, and diarrhea, primarily in the TLR4, MAPK, and apoptosis pathways. Multi-tissue analyses demonstrated SHZL's superior regulation of inflammatory (IL-6, IL-1 $\beta$ ) and apoptotic markers (BCL2, CASP8, CASP3) in the serum, heart, liver, and cecum, with targeted suppression of the TLR4/MYD88/IKK $\alpha$ /I $\kappa$ B $\alpha$ /NF- $\kappa$ B signaling pathway. Molecular docking confirmed high-affinity binding of SHZL components (coptisine, berberine) to core targets (AP-1, ERK1, IKK $\alpha$ ). Although SHZL lacked direct antibacterial activity, it enhanced gut microbial diversity and suppressed pro-inflammatory taxa, whereas oxytetracycline exacerbated dysbiosis and sustained pathogenic proliferation. SHZL established its holistic therapeutic effect through multi-functional, multi-organ, multi-composition, and multi-dimensional regulation of the host-gut microbiota axis. The multi-dimensional effects were achieved through anti-inflammatory, immunomodulatory, organ-protective, and microbiota-balancing mechanisms.

## Introduction

Colibacillosis caused by avian pathogenic *Escherichia coli* (APEC) represents a significant threat to the global poultry industry. The clinical manifestations of APEC—including diarrhea, pericarditis, perihepatitis, and intestinal lesions—cause annual economic losses exceeding billions

of US dollars (Joseph et al., 2023). While antibiotics remain the primary clinical intervention for the control of colibacillosis, the increasing resistance rates of APEC to commonly used drugs such as florfenicol and enrofloxacin and the growing concerns over drug residue-induced food safety issues have become critical challenges (Bhattarai et al., 2024; Nawaz et al., 2024). Furthermore, antibiotic treatments often

**Abbreviation:** APEC, Avian pathogenic *Escherichia coli*; SHZL, Sihuangzhili granule; TCVM, Traditional Chinese veterinary medicine; CC, *Coptis chinensis*; PC, *Phellodendron chinense*; SB, *Scutellaria baicalensis*; RP, *Rheum palmatum*; IT, *Isatis tinctoria*; GU, *Glycyrrhiza uralensis*; TLC, Thin-layer chromatography; HPLC, High performance liquid chromatography; OTC, Oxytetracycline; DAI, Disease activity index; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GGT,  $\gamma$ -glutamyltransferase; TP, Total protein; ALB, Albumin; LAC, Lactate; CHE, Serum cholinesterase; TCMSP, Traditional Chinese medicine systems pharmacology database; OB, Oral bioavailability; DL, Drug likeness; PPI, Protein–Protein interaction; GO, Gene Ontology; KEGG, Kyoto encyclopedia of genes and genomes; BP, Biological process; CC, Cellular component; MF, Molecular function; RT-PCR, Quantitative real-time PCR; ASV, Amplicon sequence variant; PCA, Principal component analysis; PCoA, Principal coordinates analysis; NMDS, Non-metric multidimensional scaling.

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