Transcriptomic Profiling of miRNA in Medicinal Herb Extracts Against Aflatoxin B1-Induced Liver Toxicity in Pigs

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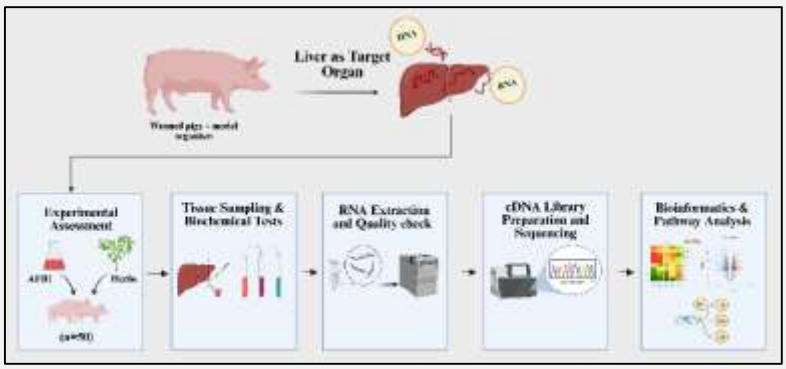
Aflatoxin B1 (AFB1) is a highly toxic compound that is known to cause significant liver damage through mechanisms involving oxidative stress, inflammation, and gene regulatory disruptions, including microRNA (miRNA) modulation. Medicinal herbs such as *Andrographis paniculata* (andrographolide), *Silybum marianum* (silymarin), and *Curcuma longa* (curcumin) are known for their liver protective effects, but their influence on miRNA regulation in cases of liver injury caused by AFB1 remains unclear. This study investigates how miRNA regulation in the pig liver transcriptome changes following AFB1 exposure and evaluates the potential hepatoprotective effects of herbal extracts using next-generation genome sequencing (NGS) techniques. A total of 50 weaned pigs were assigned into five groups (n=10 each): three experimental groups supplemented with one of the herbal extracts in combination with AFB1, a pure control group (no treatment), and an AFB1-only control group. AFB1 was administered using ethanol as a solvent mixed with 5% glucose and 9% sodium chloride, replacing traditional DMSO-based solvents to reduce unwanted interference. The study was structured over four weeks, beginning with two weeks of herbal supplementation, followed by two weeks of AFB1 exposure to evaluate potential preemptive and protective effects. Phenotypic assessments, biochemical liver function tests (ALT, AST, ALP, GGT, and PT) and hematological parameters, were conducted at different time points to determine correlations between molecular alterations, hepatotoxicity, and physiological responses.



Upon completion of the feeding experiment, liver tissues were collected from all groups for molecular analysis. Total RNA was extracted and evaluated for quality (RIN >8, A260/A280: 1.8–2.0) using the Agilent Bioanalyzer and NanoDrop spectrophotometer. Small RNA libraries were constructed using Illumina adapter ligation, reverse transcription, and PCR amplification. Library quality and integrity were assessed using Qubit™ assays, Sage Science BluePippin size selection, and Agilent Bioanalyzer with High Sensitivity DNA chips, ensuring suitability for high-throughput sequencing on the Illumina MiSeq platform. Post-sequencing analyses will include differential expression analysis and weighted gene co-expression network analysis (WGCNA) to identify miRNAs associated with liver protection. Enrichment analyses using gene ontology (GO) and pathway mapping will further clarify the regulatory roles of miRNAs in the observed hepatoprotective effects. This study establishes a well-defined experimental model to explore how herbal extract supplementation influence miRNA expression in the context of AFB1-induced hepatotoxicity. The findings are expected to provide insights into miRNA-mediated regulatory pathways and contribute to the development of RNA-based therapeutic strategies for liver protection.

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