Large Scale Isolation, Purification & Characterization of the Natural Products from *Terminalia arjuna*, and Evaluation of their Role in Metabolic Disorders

Abstract

Metabolic disorders, including Type 1 and Type 2 diabetes mellitus (DM) and nonalcoholic fatty liver disease (NAFLD), are major global health concerns driven by impaired glucose and lipid metabolism, necessitating novel therapeutics. The aim of this study was to isolate bioactive compounds on a large scale from the bark of *Terminalia arjuna*, valued in traditional medicine for managing metabolic dysfunction.

Prior to compound isolation, the safety profile of *T. arjuna* bark powder and its secondary metabolite-rich ethyl acetate extract were evaluated in animal models. Based on a pilot-scale study, *T. arjuna* dried bark powders were extracted using a Soxhlet apparatus with ethyl acetate (EtOAc). Extensive and repeated chromatographic separations on silica-based normal-phase and dextran gel-based size-exclusion chromatography yielded triterpene acid-rich fractions from the EtOAc extract. These chromatographic fractions were further purified by crystallography and preparative high-performance liquid chromatography (HPLC), yielding five major metabolites in substantial quantities, along with six minor metabolites. The five major compounds were identified using high-field nuclear magnetic resonance (NMR) experiments as arjunolic acid, arjunglucoside, arjunetin, arjunic acid, and β-sitosterol. Four of these five compounds are oleanane-type triterpene derivatives. Each of the major metabolites was isolated in quantities of more than 2.0 grams.

These isolated compounds were evaluated for therapeutic effects in animal models of Type 1 DM, Type 2 DM, and NAFLD. The efficacy as well as the molecular mechanism of the experimental compounds were examined using multiple methods. Lipid profile and hepatic enzymes were quantified using biochemical assay, and histological analysis was conducted following Hematoxylin and Eosin (H&E) and Masson's trichrome (MT) stains. The targeted proteins and mRNA expression were measured using automated Western blotting, immunohistochemistry, and RT-PCR.
The bark powder and its extract were both found safe for normal mice at three different doses (500 mg, 1g, and 2g/Kg body weight). No morbidity and mortality were found, and hematological and histological parameters were detected to be normal without any significant alteration.

In type 1 DM and NAFLD, major organ complications were also noticed along with elevation of glucose level, lipid profile, and steatohepatitis in untreated mice in comparison to the normal mice. Treatment with arjunic acid and arjunolic acid normalized these alterations at a significant level. Improvement was also noticed with arjunglucoside-1 and arjungenin treatment. Arjunolic acid ameliorated pancreatic apoptosis and necrosis, along with reducing elevated blood glucose levels in T2DM-induced rats. The expression of inflammatory markers was downregulated in arjunolic acid-treated diabetic pancreas through blocking the TLR-4/MyD88 and Wnt canonical signaling. Consistent with this data, arjunic acid, arjunoglucoside-1, arjungenin, and arjunolic acid significantly reduced the NAFLD activity score to the borderline in experimental NAFLD mice. The fibrosis was markedly reduced by the arjunglucoside-1 and arjungenin. All the treatments significantly reduced hepatic enzymes in serum and improved the pathological alterations in the heart, kidney, and pancreas, along with hepatic tissue.

In conclusion, these findings highlight the potential of isolated secondary metabolites of *T. arjuna* as novel treatments for metabolic disorders and warrant further clinical investigation with molecular mechanisms.