ABSTRACT

Tissue injury can initiate inflammation and set off a series of biochemical and physiological reactions which primarily mediate the sense of pain or other signs of inflammation. The host of cytokines and chemokines that are released as proinflammatory mediators establish a more aggressive acute phase response to injury or infection leading to conditions of chronic inflammation on prolonged stimulus. This necessitates the regulation of chronic inflammation and related conditions. Currently marketed anti-inflammatory drugs can result in deleterious side effects when used for prolonged periods of time for inflammation. Prolonged use of glucocorticoids in chronic inflammation can cause osteoporosis and muscle wasting while the popular non-steroidal anti-inflammatory drugs (NSAIDs) targeting the cyclooxygenase (COX) enzymes result in gastrointestinal ulcers, renal toxicity, ischemic cardiovascular disease, cardiac arrhythmia, and heart failure. These effects are caused as a result of shift in metabolic flux of arachidonic acid from cyclooxygenase (COX) pathway to the lipoxygenase (LOX) pathway thereby resulting in production of more potent pro-inflammatory leukotrienes. The current scenario in anti-inflammatory drug development focuses on the development of dual inhibitors of COX-2 and 5-LOX enzymes for the anti-inflammatory treatment with higher safety profile. This work focuses on the screening of medicinal plants for dual inhibitors of LOX and COX.

Plants were selected based on a two point criteria which includes reported literature on anti-inflammatory activity in adjuvant induced inflammation and use of the plant in ayurvedic or folkloric treatments for inflammation. 16 plants were selected and extracts prepared for the screening
for dual inhibition of COX and LOX enzymes resulting in 7 plants being identified with dual inhibition. The phytochemical content of these plants were analysed. The evaluation of the results and the reported use in ayurveda as an anti-inflammatory, anti-arthritic and immunomodulatory principle led to the selection of *Tinospora cordifolia* for further studies. The methanol and aqueous extracts were characterised for antioxidant free radical scavenging activity and anti-inflammatory activity.

The methanol extract was found to be comparatively more potent than aqueous extract and was further subject to bioactivity guided fractionation. The fractions were analysed for LOX inhibition and the fractions with least and most inhibitory activity was selected. These bioactive fractions were then evaluated for free radical scavenging activity, inhibition of LOX and COX isoenzymes and studying the effects of the fraction on kinetic parameter Km and Vmax of LOX isoenzymes and COX-2. The effect of *T. cordifolia* methanol extracts and bioactive fractions on the proinflammatory cytokine (TNF-α and IL-1β) production was evaluated in lipopolysaccharide (LPS) stimulated monocytic and monocyte derived dendritic cells. The production of TNF-α and IL-1β was effectively inhibited in the case of dendritic cells than monocyte cells.

The bioactive molecules in the active fractions and methanol extract were identified with the use of UPLC-Q-ToF MS/MS system leading to the identification of 12 structures. The mass peaks at 418, 469 and 523 were found commonly in all fractions. The mass error for molecular ions of all identified compounds was within ±6 ppm. The bioactive molecules identified were further analysed by molecular docking studies with the 5-LOX and COX-2 enzymes. The software used was the Schrodinger suite with Maestro version 9.3. The Glide (Grid-based Ligand Docking with Energetics)
program was used for the docking studies in the standard precision mode. The results were given in G score (binding energy) and the hydrogen bonding was also studied. The presence of neighbouring amino acids between receptor and ligands were also evaluated. The two best docked ligand with lowest Glide score value was selected as the most effective molecules. The identified bioactive molecules could serve as lead compounds for further studies in the development of anti-inflammatory compounds.