Abstract

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Thesis title: "Studies on lipid-induced mitochondrial dysfunction and associated immune-metabolic perturbations: an approach towards novel therapeutic interventions"

Introduction:

In recent time sedentary lifestyle, poor food habits, and reduced physical activity are leading cause towards increase in obesity, insulin resistance, and dyslipidemia. Western diets, saturated fats, and adulterated oil contribute to the accumulation of free fatty acids inside hepatic cells, causing hypertension, mitochondrial dysfunction, and type-2 diabetes. Nonalcoholic fatty liver disease (NAFLD) affects over 30% of the general population and if untreated progresses to steatohepatitis, fibrosis, cirrhosis, and end-stage hepatocellular carcinoma. *Wrightia tinctoria*, also known as Indrajao, is used in traditional medicine for controlling jaundice, hyperglycemia, bowel diseases, and psoriasis. This study aimed to investigate the potential of *Wrightia tinctoria* seed extracts in improving lipid stress-induced metabolic diseases like NAFLD and associated mitochondrial anomalies.

Methodology:

The study used palmitic acid as a lipid stress inducer in an in-vitro model using hepatoma (HepG2), adipocyte (3T3L1), and macrophage (RAW264.7) cell lines. Phyto-extracts were extracted using various solvents (Hexane, ethanol, 50:50 ethanol: water and water). Molecular biology techniques like quantitative PCR, western blotting, and confocal microscopy were used. Mitochondrial morphology and integrity were studied using fluorescent probes such as Mito tracker red. Extracellular flux analyzers were used to measure mitochondrial bioenergetics parameters. The extract was then orally administered for 28 days to mice fed on a high fat diet (20weeks), and molecular experiments were conducted to validate its effect on mitochondrial dysfunction.

Results:

Based on the screening of four extracts, we can see the (50: 50) hydro-ethanolic extract (WTEC) of *Wrightia tinctoria* seedpod did not exhibit any cytotoxicity on human liver cell line in a broad range of concentration as well as in *in vivo* acute oral toxicity experiments. Consistently this fraction showed significant antioxidant activity and hindered lipid accumulation upon giving high saturated fatty acid stress and also improved mitochondrial bio-energetic function. This fraction along with hexane fraction (Fr-B) from ethyl acetate extract also effectively lowered pro-inflammatory markers often associated with NAFLD progression. WTEC significantly

boosted mitochondrial biogenesis through activation of PGC 1α, SIRT 1 and TFAM expression. Mitochondrial dynamics was also improved by inhibition of phosphor-DRP1 (Serine 616) mediated mitochondrial fragmentation. Wrightia seedpod extract also activated mitophagy maintaining quality control. PARKIN and PINK1 level was restored and LC3B expression was augmented in palmitic acid treated hepatoma cells. Lipid metabolism was also boosted as PPARα, CPT1A level increased and fatty acid uptake was down by lowered CD36 level. WTEC also alleviated abnormal lipid levels, fasting blood glucose and collagen deposition in HFD model of NAFLD improving gross hepatic steatosis. Thus, WTEC at 1to 10 µg/ml dose range *(in vitro* hepatoma cells) and 250 -500mg/kg bodyweight (in *vivo* animal model) efficiently reversed the distressed mitochondrial physiology imparted by lipotoxic stress.

Conclusion:

Wrightia tinctoria is well acknowledged herb used in many traditional and ayurvedic treatment. However, the detailed mechanistic study on the mechanism of action of its specific extracts responsible for its positive effects, are still lacking. After comparing different extracts of wrightia seeds, the hydro-ethanolic extract (WTEC) showed the most potential beneficial effect in ameliorating the deregulated metabolic parameters. This particular extract improves the overall mitochondrial health as shown in hepatic lipotoxicity model employing palmitic acid and high fat diet, making it a safe and potential candidate against NAFLD. Characterization of compounds revealed presence of ω-3 poly unsaturated fatty acids (PUFA) in hexane fraction and phenolic compounds in WTEC, mainly responsible for bio-activity. Thus, wrightia seedpods hold an immense potential to generate an effective therapeutic lead against lipid-induced mitochondrial dysfunction and associated immune-metabolic perturbations in diseases like NAFLD.

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