

METABOLIC CHANGES ASSOCIATED WITH THE EFFECT OF *Morinda citrifolia* L. LEAF EXTRACT IN THE PREVENTION AND TREATMENT OF OBESITY IN SPRAGUE- DAWLEY RATS

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The prevalence of obesity is increasing worldwide, both in developed and developing nations and a high fat diet is one of the main factors. It is known that obesity also increase the predisposition to other diseases such as diabetes and cardiovascular diseases through the involvement of various metabolic pathways. The lack of anti-obesity drugs and the popularity of alternative and complementary medicine has encouraged research in finding phytochemical strategies to this multifaceted disease. In this study, extracts of *Morinda citrifolia* leaf of different maturity and extracted with different ethanol concentrations were assessed for their bioactivity (total phenolic content, DPPH scavenging activity, inhibitory effects on pancreatic and lipoprotein lipase activity) as potential anti-obesity agents. Mature leaves extracted with 60% ethanol, labeled as MLE 60 had the highest lipase inhibitory effect and was further analysed for its bioactive content using ¹H Nuclear Magnetic Resonance spectroscopy (¹H NMR), Liquid Chromatography Mass Spectroscopy (LCMS) and High Performance Liquid Chromatography (HPLC). MLE 60 was found to contain flavonoids, including catechin, kaempferol and rutin. The anti-obesity effect of the same extract was subsequently assessed for the prevention and treatment of obesity, *in vivo*. In the preventive study, lean Sprague- Dawley rats were fed a high fat diet (HFD) with or without MLE 60 for 12 weeks and assessed for weight gain, adiposity, appetite, fecal fat excretion and plasma biochemistry. Supplementation of MLE 60 in the HFD group prevented weight gain (98.6-129.6%) as compared to the control HFD only group (176.3%), reduced adiposity (3.45-4.04%) as compared to the control obese group (6.98%), increased fecal fat excretion (11.39-19.58%) compared to 5.34% for the control group, without any effect on appetite. The plasma biochemistry profiles were improved, with a marked decrease in total triglycerides, leptin and insulin levels. For the treatment study, HFD induced obese Sprague- Dawley rats were treated with MLE 60 post feeding with a HFD for 12 weeks. Similar parameters as the preventive study were measured. After 9 weeks of treatment, no significant weight loss was achieved in any of the treated group, including the group treated with the standard control drug Orlistat[®], though positive effects were observed on adiposity, fecal fat content, plasma lipids, insulin and leptin levels. The inducement of obesity and treatment with MLE 60 on metabolic alterations were then further elucidated using a ¹H NMR based metabolomics approach, where the urine and serum of obese and lean rats were compared for biomarkers associated with HFD induced obesity. Multivariate analysis, including the projections to latent structures-

discriminant analysis (OPLS-DA) was used for biomarkers identification. Discriminating metabolites involved were products of various metabolic pathways, including glucose metabolism and TCA cycle (lactate, 2-Oxoglutarate, citrate, succinate, pyruvate, acetate), amino acid metabolism (alanine, 2-hydroxybutyrate), choline metabolism (betaine), creatinine metabolism (creatinine) and gut microbiome metabolism (hippurate, phenylacetylglutamate, dimethylamine, trigonelline). Treatment with MLE 60, specifically at 250 mg/kg, resulted in significant improvement in the metabolic perturbations caused by HFD induced obesity as demonstrated by the proximity of the treated group to the normal group in the OPLS-DA score plot and the change in trajectory movement of the diseased group towards the healthy group upon treatment. A relative quantification of discriminating metabolites showed improvements in the treated groups. This study reports on the potential anti-obesity effect of MLE 60, based on its lipase inhibiting ability as reflected as by the increase fecal fat content and its positive effect on pro obesity factors including leptin and insulin. It also confirms that consumption of a HFD caused metabolic perturbations other than traditionally studied parameters, which can be improved by the supplementation of plant extracts like MLE 60 and ¹H NMR based metabolomics can be a good tool in obesity research.