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Ellagitannin geraniin supplementation ameliorates metabolic risks in high-fat diet-induced obese Sprague Dawley rats

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ABSTRACT

Geraniin, an ellagitannin found abundantly in many fruits, nuts, traditional Chinese medicine (TCM) and ayurvedic herbs, has been reported to possess numerous health benefits. This is the first study that elucidates geraniin, purified from the sub-tropical fruit *Nephelium lappaceum* L. rind, for its therapeutic potential in ameliorating diet-induced metabolic risks mimicking metabolic syndrome. Male post-weaning outbred Sprague Dawley rats received a 60% high-fat diet (HFD), with and without the geraniin supplementation (10 and 50 mg/kg body weight), while the control group (ND) was fed rat chows for 10 consecutive weeks. Comparatively, HFD rats demonstrated elevated body weights, white adipose tissue depots (WAT), organ weights, triaylglycerol, renal and hepatic dysfunction biomarkers, insulin resistance, declined insulin sensitivity and percent of beta-cell function. A four-week *in vivo* geraniin treatment, particularly at 50 mg/kg body weight, exhibited significant therapeutic potential to safely mitigate obesity-induced metabolic dysfunction.

1. Introduction

Excess nourishment, high fat, sugar and salt in modern diets, combined with a sedentary lifestyle, leads to metabolic overload and consequently metabolic disturbance, which is an imbalance between energy input (EI) and energy output (EO) (Sorensen, 2009). It results in over-abundance of glucose and fatty acid accumulations within adipose tissue, skeletal muscle, hepatocytes and pancreatic cells, causing serious defects in fuel partitioning and thus disrupts the total body energy homeostasis (Baur et al., 2006; Chen et al., 2011; Storlien et al., 1996), finally leading to the risks associated with metabolic syndrome (MS). Metabolic syndrome is typified by a constellation of metabolic risks encompassing obesity, insulin resistance, hyperglycaemia, dyslipidaemia and hypertension (American Diabetes Association, 2001; Christopher & Sarah, 2005; National Heart, Lung and Blood Institute, 2001). The number of people affected with metabolic syndrome worldwide has strikingly increased over the past two decades and this increase is not dissociable from the worldwide pandemic of obesity and hyperglycaemia (Zimmet, Alberti, & Shaw, 2001). Many studies have shown that lifestyle interventions including increased physical activity, dietary modification and

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weight management in combination with pharmacotherapy reduce the incidence of these metabolic risks (American Diabetes Association, 2001; Christopher & Sarah, 2005; National Heart, Lung and Blood Institute, 2001). However, there are still difficulties in reaching the goal of normalising glucose levels and fat contents without adverse effects, such as severe weight gain or loss, hypoglycaemic episodes, lactic acidosis, hepatoxicity, kidney damage, dyspepsia, atherogenic events and premature cardiovascular diseases due to hyperinsulinaemia and lipodystrophy, overstimulation and appetite abuse in addition to raised risk of death (Agabegi & Steven, 2008; Zimmet et al., 2001). Hence, there is a great medical need to develop novel drugs or alternative therapies that are both effective and which are free from, or with relatively fewer adverse effects.

Traditionally, plants have been used in the management of a broad spectrum of metabolic dysfunction such as diabetes, cardiovascular diseases, obesity, dyslipidaemia and cancers, among others, owing to the presence of plant-derived secondary metabolites (Aggarawal & Shishu, 2011). Plant-derived secondary metabolites are a rich source of natural bioactive components; the most essential of these are flavonoids and polyphenols. Ellagitannins (ETs), the bioactive polyphenols, can be found abundantly in fruits such as raspberries, blackberries, strawberries, cranberries and pomegranates; in vegetables such as potato, tomato, lettuce and onion; in nuts and seeds (Scalbert & Williamson, 2000), traditional Chinese medicinal plants such as *Geranium sibiricum* Linne (Yang et al., 2010) and ayurvedic herbs such as *Phyllanthus emblica* (amla) (Krishnaveni & Mirunalini, 2010). Many studies on ellagitannins have demonstrated positive biological actions encompassing antioxidant, antimutagenic, anticarcinogenic, antitumour, antiviral and antimicrobial, both *in vitro* and *in vivo* (Ito, 2011), which suggest that the consumption of ellagitannins may provide protective benefits on human health.

Native to Southeast Asia, *Nephelium lappaceum* L. ('rambutan' in Malaysia language) belongs to the same family (Sapindaceae) as the sub-tropical fruits lychee (*Litchi chinensis*) and longan (*Dimocarpus longan*). This fruit is an important commercial crop in Asia, where it can be consumed fresh, canned or processed. Geraniin, a typical ellagitannin was recently established to be present in *N. lappaceum* L. rind with the highest yields of geraniin (up to 35%) among the plant studied thus far (Palanisamy, Ling, Manaharan, & Appleton, 2011). Studies on diverse biological properties of geraniin have shown that the compound exhibits antihyperglycaemic potential (Palanisamy et al., 2008), antihypertensive activity (Lin, Wang, Lu, Wu, & Hou, 2008), hepatoprotective action (Ambrose, Solairaj, & Subramoniam, 2012), high antioxidant (Palanisamy et al., 2008; Thitilertdecha, Teerawutgulrag, Kilburn, & Rakariyatham, 2010) and nitrogen oxide (NO) scavenging capacity (Kumaran & Karunakaran, 2006). Previous *in vitro* investigations on 3T3-L1 cells have also revealed geraniin's ability to enhance glucose uptake (Palanisamy). Based on its manifold beneficial health properties, geraniin is a valuable candidate for more extensive study into its potential pharmaceutical applications. This study investigates the *in vivo* effects of supplementing geraniin purified from the *N. lappaceum* L. rind on metabolic factors mimicking MS and its clinical complications in rodents fed on a high-fat diet.

2. Materials and methods

2.1. Materials

High-fat pellet (HFD, 60% fat by weight, AIN93G specifications purified diet) was purchased from Specialty Feeds Inc. (Glen Forrest, Western Australia). Normal rat chow (ND, 5% minimum crude fat content) was purchased from Gold Coin (Kuala Lumpur, Peninsular Malaysia). Accu-Chek®Performa Glucometer was purchased from Roche (Manheim, Germany). Diagnostic reagents for metabolic parameter measurements of lipids (TG, TC, HDL and non-HDL cholesterols), liver (ALT, AST and GGT) and kidney (CK and Cr) were purchased from Roche (Manheim, Germany). Rat/Mouse Insulin Sandwich ELISA kit was purchased from Millipore (MA, USA). BIO-RAD Benchmark Plus Microplate Reader with Microplate Manager 5.2.1 software (CA, USA), Hitachi Model 902 Automatic Analyser (Manheim, Germany). All other chemicals and reagents were purchased from Becton, Dickinson and Company (NJ, USA), Millipore (MA, USA), Sigma-Aldrich (MO, USA), Terumo (Tokyo, Japan) and Vétoquinol UK Limited (Buckingham, UK) unless stated otherwise.

2.2. *Preparation of geraniin from* Nephelium lappaceum *L. rind by reverse phase C-18 column chromatography*

Nephelium lappaceum L. was obtained from Kuala Lumpur, Peninsular Malaysia. Plants were authenticated by the Herbarium of the Forest Research Institute of Malaysia (FRIM). Crude extract of *N. lappaceum* L. rind was prepared as described by Palanisamy et al. (2008). Geraniin was purified from the crude extract by means of reverse-phase C-18 chromatography (Palanisamy et al., 2011). Crude extract (20 g) was dissolved in a minimum amount of water (40 mL) and loaded onto glass column (250 mm × 50 mm i.d.) packed with 200 g C18 silica (particle size of 50 μm, pore size of 60 Å). The column was open tubular and solvent flow rate was maintained by means of vacuum pump attached to vacuum inlet in the column. The column was first eluted with water (300 mL) and then fractions were collected using a step gradient of water and acetonitrile. Solvent system was as follows: water (100%, 400 mL), acetonitrile/water (5:95, 350 mL), acetonitrile/water (10:90, 1000 mL). Finally the column was eluted with methanol (100%, 500 mL). The silica was cleaned by flushing the column sequentially with dichloromethane (100%, 300 mL), methanol containing a few drops of trifluoroacetic acid (100%, 300 mL) and absolute methanol (100%, 300 mL), allowing to dry completely. This enables the column to be reused with fresh crude extract. Purity of geraniin (> 95%) obtained was confirmed using HPLC (Details see Perera, Appleton, Loh, Elendran, & Palanisamy, 2012).

2.3. *Animals and housing*

Thirty-two male, post-weaning (3-week-old) outbred Sprague Dawley (SD) rats (*Rattus norvegicus*) were obtained from the Animal House of Monash University (Monash University, Peninsular Malaysia). Males were used to eliminate variations in