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Lavatera critica controls systemic insulin resistance by ameliorating adipose tissue inflammation and oxidative stress using bioactive compounds identified by GC–MS

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ABSTRACT

Background: *Lavatera critica*, a leafy green herb, is reported to have many pharmacological activities; but, the improvement of insulin sensitivity against the high gram-fat diet (HGFD)-caused insulin resistance (IR) has not yet been studied.

Objective: This study evaluated the role of *Lavatera critica* leaf extract (LCE) in systemic insulin resistance through the alleviation of adipose tissue inflammation and oxidative damage in HGFD fed mice.

Methods: The mice were fed with HGFD for 10 weeks and the diet was supplemented with LCE each day for the next five weeks. Body weight, food intake, leptin, blood glucose, insulin, insulin resistance, and pro- and anti-inflammatory genes expression were assessed on day 106.

Results: The HGFD control mice displayed markedly elevated adipose tissue inflammation, oxidative stress, insulin inactivity, and hyperglycemia. Administration of LCE in the HGFD mice, especially a dose of 100 mg/kg, lowered the body weight, food intake, plasma leptin, plasma glucose, plasma insulin, insulin resistance, and increased the food efficacy ratio when compared with the HGFD control mice. The oral glucose tolerance test (OGTT) revealed that LCE prevented further increase in the circulating levels after the glucose load. LCE-treated mice demonstrated a marked suppression of pro-inflammatory cytokines mRNA expression. On the other hand, the mice showed a higher anti-inflammatory genes mRNA expression in the adipose tissue. In addition, LCE treatment improved the oxidative damage as evidenced by the reduced levels of lipid hydroperoxides and thiobarbituric acid reactive substances coupled with the increased antioxidants (superoxide dismutase, total glutathione, glutathione/glutathione disulfide ratio and glutathione peroxidase) in the adipose tissue, plasma and erythrocytes. Gas chromatography-mass spectrometry analysis of the bioactive compounds revealed the presence of 9, 12, 15-octadecatrienoic acid, vitamin E, phytol, hexadecanoic acid, benzenepropanoic acid, and stigmasterol.

Conclusions: These findings prove that LCE improves the insulin-sensitizing activity in the mouse model of HGFD-caused IR, probably due to the amelioration of adipose tissue inflammation and oxidative damage. Hence, the LCE could serve as a useful anti-diabetic agent.

1. Introduction

Obesity is a global health concern and its prevalence continues to increase in developing as well as developed countries due to less healthy lifestyle practices and reduced physical activity. Chronic obesity is a central factor for the occurrence of type 2 diabetes (T2D), hypertension, and metabolic diseases [1]. Prolonged utilization of a HGFD leads to obesity, IR, hyperglycemia, and metabolic disorders in experimental animals [2]. Insulin binds to a specific cell surface receptor which activates a numeral of protein kinases concerned in the signaling pathway. IR is a pathological condition resulting from an impairment of this pathway; the target cells fail to respond to the circulating hormone, which in turn affects the entire signaling cascade.

Adipose tissue chronic inflammation plays a primary role in the development of IR and hyperglycemia in mice fed with a HGFD for a long duration [3]. Obesity, particularly excess visceral adiposity, is mainly implicated in IR and T2D [4]. Prolonged absolute low-grade inflammation is associated in acquiring obesity-related T2D and metabolic disorders [5]. Chronic obesity causes inflammation in the adipose tissue and additionally affects the liver, skeletal muscle, and pancreas.

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Specifically, the adipose tissues secrete pro-inflammatory cytokines and adipokines, which prevent the uptake of insulin in the skeletal muscle and liver, thereby resulting in comprehensive IR [6].

Recent clinical and animal evaluations have established the connection between the oxidative damage and obesity. Further, the risk factors have also been analyzed [7]. Oxidative damage can cause to obesity by altering the food intake and stimulating the deposition of excess fat in the adipose tissue. Besides, the condition has pertinent pathologic roles in increasing the adipocyte size, proliferation, and differentiation [8–10]. Reactive oxygen species (ROS) are imperative in controlling the body weight due to their influence on the hypothalamic neurons which regulate hunger and satiety [11]. Obesity is also related to the oxidative damage that occurs through biochemical mechanisms including, enhanced NADPH oxidase activity, chronic inflammation, glyceraldehyde auto-oxidation, oxidative phosphorylation, and decreased anti-oxidant defenses [7,12–14].

The preceding sections highlight the importance of preventing chronic inflammation and oxidative stress in dealing with obesity-associated IR. Numerous studies have reported that natural sources provide protection against obesity-associated IR by attenuating inflammation and oxidative damage [15]. Despite the existence of standard anti-diabetic drugs in the medico-market, research on medicinal plants for the development of alternative anti-diabetic therapies is successful around the world. Plant-based extracts contain innovative primary and secondary metabolites, such as phenolic acid, terpenoids, alkaloids, steroids, and flavonoids. Such extracts are notable therapeutic options for improving insulin sensitivity due to their non-toxic, cost-effective, and eco-friendly nature. Recent studies have proved that certain medicinal plants improve glucose tolerance and have anti-diabetic effects related to the regulation of hyperplasia/adipogenesis and glucose uptake [16]. Thus, the discovery of natural compounds that treat insulin resistance has become an attractive area of research. It is worth noting that the HGFD administration to C57BL/6 J mice employed in this study is the most common and useful animal model to estimate the property of plant extracts on insulin activity. *Lavatera cretica* L. (common name: Cornish mallow, Arabic name: Khobiza) leaf selected for this research is consumed by the Saudi Arabian people and is a natural, inexpensive, and environment-friendly resource. The ability of *L. cretica* to regulate diabetes is still unclear; therefore, the present study evaluated its efficacy in controlling the impaired systemic insulin sensitivity and oxidative damage caused by inflammation.

L. cretica belongs to the *Malvaceae* family [17]. The plant is a well-known leafy green vegetable that is widely cultivated in Saudi Arabia, and it has been used as a folk medicine in certain regions to treat influenza and digestive problems. Moreover, due to its antitussive, laxative, and anti-inflammatory properties, the herb is applied in some areas of Spain for curing the aforesaid maladies, as well as upper respiratory tract disorders [18]. Only a less studies have explored the medicinal properties of *L. cretica*. Viegi et al. have described the utilization of its extract as a veterinary medicine to treat gastrointestinal illnesses in cattle [19]. Additionally, the plant displays potential antioxidant and anti-lipoxygenase activities [20].

2. Materials and methods

2.1. Animals

Five weeks of Male C57BL/6 J mice were used for all experiments. The mice were received from King Saud University Central Animal House and housed in polypropylene cages. The mice were maintained at room temperature (22 ± 2 °C) with a 12-h day/night cycle. The animal care guidelines conformed to the regulations of King Saud University Research Centre and the study was approved by the centre.

2.2. Extraction of L. cretica leaves

Fresh *L. cretica* leaves were congregated from the Riyadh region, Saudi Arabia. The plant was authenticated at the King Saud University Botany Department. The leaves were cleaned of dust with running tap water followed by triple distilled water. Cleaned leaves were dried in the shade at room temperature for 2 days and powdered using a blender. A 10 g portion of the grinded powder was floated in n-hexane (300 ml) for 24-h. The n-hexane extract was decocted by passage through Whatman filter paper and concentrated at 35 ± 40 °C. The concentrated extract was stored until used for further experiments.

2.3. Identification of compounds by gas chromatography-mass spectrometry (GC–MS)

The organic chemical constituents of *L. cretica* were identified by GC–MS using a model 7890 A device and MS5975 system (Agilent, USA). The GC–MS system was outfitted with a 30 m × 0.25 mm silica column containing 5% diphenyl and 95% dimethylpolysiloxane. Helium is a carrier gas that was used in constant flow rate (1 mL/min). 70 eV of electron ionization energy was programmed for this analysis. The oven temperature was fixed at 50 °C initially for 4 min. after it was increased stepwise. The crude extract was diluted with high-performance liquid chromatography (HPLC) grade methanol and filtered. The diluted free particle extract was injected into the GC injector using a syringe. The total running time was 35 min. *L. cretica* extract compounds were identified by comparing the GC retention peaks with standards in the National Institute of Standard Technology database library (NIST-11).

2.4. Experimental induction of diabetes

Five weeks of Male C57BL/6 J mice were maintained under standard conditions during 1 week of acclimatization. A normal pellet diet and ad libitum water were provided during the acclimatization. After the acclimation, the same diet was continued in some mice and others received the HGFD consisting of high gram fat (beef tallow-associated fat) (Table 1). The diets were continued for 10 weeks. After 10 weeks, blood glucose was determined in mice in the HGFD group. The HGFD resulted in the development of diabetes, as confirmed by a blood glucose level that always exceeded 180 mg/dL.

2.5. Oral glucose tolerance test (OGTT) (after 10 weeks or prior to the LCE long-term (five weeks) treatment period)

After 10 weeks, either the normal diet or the HGFD induced insulin resistance mice were separated into five groups (Four mice per group). Group 1 was a normal group consisting of mice fed with normal diet. Groups 2–5 comprised mice fed with the diabetes inducing HGFD. All the groups were used to determine OGTT. The mice were fasted prior to the OGTT for 6 h. For the test, 2 g/kg of glucose was orally administered to all the mice. Immediately thereafter, LCE (50, 100 and 200 mg/kg)

Table 1
Constituents of normal gram and high gram-fat diet.

Ingredients	Normal gram fat diet (g/100 g)	High gram-fat diet
Protein	21.1	21.1 g/100 g
Fat	5.1	5.1 g/100 g
Carbohydrate	60.0	60.0 g/100 g
Fiber	3.9	3.9 g/100 g
Minerals	7.9	7.9 g/100 g
Vitamins	2.0	2.0 g/100 g
Beef tallow	———	34.9 g mixed with 100 g of standard pellet diet