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ORIGINAL ARTICLE

Grape seed and skin extract as an adjunct to xenical therapy reduces obesity, brain lipotoxicity and oxidative stress in high fat diet fed rats

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Obesity;
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Summary
Background: Obesity is a public health problem and a major risk factor for metabolic syndrome. This study was designed to assess the effectiveness of grape seed and skin extract (GSSE) and Xenical (Xe) on high fat diet (HFD)-induced obesity and brain lipotoxicity.
Method: Rats were rendered obese and then treated either with vehicle (control) or GSSE (4 g/kg bw) or Xe (1, 2, 4 or 8 mg/kg bw) or (GSSE + Xe) and monitored for weight loss during 3 months. Animals were then sacrificed and their brain utilised for the evaluation of lipotoxicity-induced oxidative stress as well as the putative protection offered by GSSE and Xe treatment.
Results: As expected HFD-induced body and adipose tissue weight gain, dyslipidemia, accumulation of lipid into the brain, a drop in adiponectin, increased oxidative stress and disruption of Mn, Ca^{2+} and of related enzyme activities as glutamine synthetase and calpain. Xe alone exerted anti-obesity effect during the first 2 months and became inefficient thereafter. GSSE per se exhibited potent anti-obesity effect whereas the combination (GSSE + Xe), by acting in concert, was the most efficient against obesity and brain lipotoxicity. GSSE acted partially through its anti-oxidative properties, whereas Xe did not.

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Conclusion: Combining GSSE with Xe improved outcomes in body weight and fat reduction as well as in brain lipotoxicity.

Introduction

Obesity is a global health concern characterised by an imbalance between energy intake and expenditure [1] leading to the accumulation of adipose tissue. Obesity is a risk factor for the development of metabolic syndrome disorders such as type 2 diabetes, hypertension, dyslipidemia, atherosclerosis. Obesity-induced lipotoxicity refers to an ectopic accumulation of lipid in non-adipose tissues as heart, liver, kidney, pancreas, lung and also the brain contributing to organ injuries in the context of metabolic diseases [2]. Lipotoxicity is characterised by an oxidative stress within the brain, which is implicated in a wide range of pathologies including neurodegenerative diseases and ageing [3].

Up to now, many therapeutic strategies have been developed to prevent and treat obesity, among which the use of pharmacological agent as orlistat (Xenical^{TM}) which is the standard anti-obesity drug approved by US FDA for long term management of obesity [4].

GSSE is a complex polyphenolic mixture [5] exhibiting antioxidant and anti-inflammatory properties [6,7]. Grape polyphenols exerted wide range beneficial health effects, including the reduction of cardiovascular diseases [8], obesity [9], hypertension [10], dyslipidemia [11]. Grape seed polyphenols have been recently shown to protect the brain in various experimental settings including cafeteria diet-induced obesity [12], ischaemia reperfusion injury [13] and AD transgenic mice model [14].

The aim of the current study was to determine whether Xe and GSSE, either alone or in combination, were able to modulate HFD-induced obesity and brain lipotoxicity.

Material and methods

Reagents and diets

Grape seed and skin extract (GSSE) was obtained from a grape cultivar (Carignan) of *Vitis vinifera* from northern Tunisia. Seeds were manually sorted from skin, air dried and grounded separately with a coffee grinder. Both powders were then mixed at 50:50 ratio on a dry weight basis in 10% ethanol (v/v). After vigorous stirring and centrifugation at 10 000 $\times$ *g* for 15 min at 4 °C, supernatant containing soluble polyphenols was daily administered to animals.

Xenical (orlistat; (S)-2-formylamino-4-methyl-pentanoic acid (S)-1-[[(2S, 3S)-3-hexyl-4-oxo-2oxetanyl] methyl]-dodecyl ester) was obtained from Pharmalpa, France. Standard diet (SD) was purchased from ALMAS, Bizerte, Tunisia. High-fat diet (HFD) was prepared by soaking commercial food pellets into warmed and liquefied fat from animal origin (sheep) during 15 min and allowed to dry at room temperature.

GSSE composition

Total phenolic content was determined by the Folin-Ciocalteau colorimetric method [15]. Flavonoids were determined according to Dewanto et al. [16] and condensed tannins according to Sun et al. [17]. Polyphenol composition of GSSE was determined by HPLC—MS/MS [18].

Animals and treatment

Fifty-four male Wistar rats from Pasteur Institute (Tunis) were used in agreement with the NIH guidelines [19]. They were maintained in animal facility at controlled temperature (22 $\pm$ 2 °C), a 12-h light/dark cycle for 6 months. Rats were randomly allocated into nine groups and daily treated by intraperitoneal (i.p.) way as follows:

- Group 1 (control): Rats fed SD during 6 months and administered with 10% ethanol during the last 3 months (n = 6).
- Group 2 (GSSE): Rats fed SD during 6 months and treated with 4000 mg/kg bw GSSE during the last 3 months (n = 6).
- Group 3 (HFD): Rats fed HFD during 6 months and administered with 10% ethanol during the last 3 months (n = 6).
- Group 4 (HFD + GSSE): Rats fed HFD during 6 months and treated with 4000 mg/kg bw GSSE during the last 3 months (n = 6).

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