Preventive effects of *Salvia officinalis* leaf extract on insulin resistance and inflammation in a model of high fat diet-induced obesity in mice that responds to rosiglitazone

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ABSTRACT

Background. *Salvia officinalis* (sage) is a native plant to the Mediterranean region and has been used for a long time in traditional medicine for various diseases. We investigated possible anti-diabetic, anti-inflammatory and anti-obesity effects of sage methanol (MetOH) extract in a nutritional mouse model of obesity, inflammation and insulin resistance, as well as its effects on lipolysis and lipogenesis in 3T3-L1 cells.

Methods. Diet-induced obese (DIO) mice were treated for five weeks with sage methanol extract (100 and 400 mg kg^{-1}/day bid), or rosiglitazone (3 mg kg^{-1}/day bid), as a positive control. Energy expenditure, food intake, body weight, fat mass, liver glycogen and lipid content were evaluated. Blood glucose, and plasma levels of insulin, lipids leptin and pro- and anti-inflammatory cytokines were measured throughout the experiment. The effects of sage MetOH extract on lipolysis and lipogenesis were tested *in vitro* in 3T3-L1 cells.

Results. After two weeks of treatment, the lower dose of sage MetOH extract decreased blood glucose and plasma insulin levels during an oral glucose tolerance test (OGTT). An insulin tolerance test (ITT), performed at day 29 confirmed that sage improved insulin sensitivity. Groups treated with low dose sage and rosiglitazone showed very similar effects on OGTT and ITT. Sage also improved HOMA-IR, triglycerides and NEFA. Treatment with the low dose increased the plasma levels of the anti-inflammatory cytokines IL-2, IL-4 and IL-10 and reduced the plasma level of the pro-inflammatory cytokines IL-12, TNF-α, and KC/GRO. The GC analysis revealed the presence of two PPARs agonist in sage MetOH extract. *In vitro*, the extract reduced in a dose-related manner the accumulation of lipid droplets; however no effect on lipolysis was observed.

Conclusions. Sage MetOH extract at low dose exhibits similar effects to rosiglitazone. It improves insulin sensitivity, inhibits lipogenesis in adipocytes and reduces inflammation as judged by plasma cytokines. Sage presents an alternative to pharmaceuticals for the treatment of diabetes and associated inflammation.

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Additional Information and Declarations can be found on page 18

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ORIGINAL ARTICLE

Comparison of potential preventive effects of pomegranate flower, peel and seed oil on insulin resistance and inflammation in high-fat and high-sucrose diet-induced obesity mice model

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Abstract

Objective: The potentially beneficial effects of pomegranate peel (PPE), flower (PFE) and seed oil (PSO) extracts, in comparison with rosiglitazone, on adiposity, lipid profile, glucose homoeostasis, as well as on the underlying inflammatory mechanisms, were examined in high-fat and high-sucrose (HF/HS) diet-induced obese (DIO) mice. *Measurements*: Body weight, body fat, energy expenditure, food and liquid intake, blood glucose, and plasma levels of insulin, lipids and cytokines were measured. *Results*: After two weeks, PSO (2 ml/kg/day) and rosiglitazone (3 mg/kg/day) had not improved glucose intolerance. After 4 weeks, both treatments significantly reduced fasting blood glucose and an insulin tolerance test showed that they also improved insulin sensitivity. Treatment with PPE, PFE and PSO, reduced the plasma levels of the pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α), and PFE increased the level of the anti-inflammatory cytokine interleukin-10 (IL-10). *Conclusion*: PPE, PFE and PSO have anti-inflammatory properties. PSO also improved insulin sensitivity.

Keywords

High-fat/high-sucrose diet, inflammation, insulin sensitivity, pomegranate flower, pomegranate seed oil

History

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Introduction

The world is witnessing an increase in the prevalence of obesity in almost all countries (Al-Muammar & Khan, 2012). Obesity is linked to several diseases, such as type-2 diabetes and cardiovascular disorders, characterized by hypercholesterolaemia and hypertension, insulin resistance and altered lipid metabolism (Roberts *et al*., 2004; Yang *et al*., 2012). The global prevalence of diabetes has increased over the past 18 years from 135 million in 1995 to 382 million in 2013. It has been projected that the prevalence in 2025 will be 300 million (Guariguata *et al*., 2014).

The excessive intake of high-fat and high-sucrose (HF/HS) diets is one of the primary causes of obesity and related metabolic disorders (Hulman & Falkner, 1994). A HF/HS diet is also associated with an increase in the expression of genes involved in lipid accumulation and inflammation in liver (Yang *et al*., 2012). In general, the development of obesity is associated with insulin resistance. Normal β-cells compensate for insulin resistance by increasing insulin secretion, but insufficient compensation leads to glucose intolerance and eventually type-2 diabetes.

Alternative medicines and natural therapies offer a potential treatment for type-2 diabetes but without the prominent side effects of some synthetic drugs. Many traditional plant treatments for diabetes are used throughout the world (Bailey & Day, 1989). The ethnobotanical information reports about 800 plants that may possess antidiabetic properties (Alarcon-Aguilara *et al*., 1998).

Punica granatum (pomegranate) is a large shrub distributed throughout the Mediterranean basin and the Middle East. Its health-promoting effects have been identified from several studies. It is a rich source of natural compounds, such as phenolics, which act as natural antioxidants (Melo *et al*., 2014) and can reduce the risk of major chronic diseases.

Pomegranate peel (PP) has been used traditionally for its antioxidant and anti-inflammatory medicinal properties (Al-Muammar & Khan, 2012). It enhances the free-radical scavenging activity of hepatic enzymes and reduces lipid peroxidation (Singh *et al*., 2002). It also modulates the gut microbiota by downregulating the expression of key inflammatory genes, such as interferon-γ (IFN-γ), tumour necrosis

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Metabolic effects of non-psychoactive cannabinoids in animal models

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Introduction

Two non-psychoactive cannabinoids tetrahydrocannabivarin (THCV) and cannabidiol (CBD) have been examined in a variety of animal models of obesity, diabetes and the metabolic syndrome. THCV is a neutral CB-1R antagonist and, unlike the various synthetic agents such as rimonabant, which is an inverse agonist, it potently blocks natural agonists in *in vitro* preparations. CBD is a putative antagonist of GPCR55.

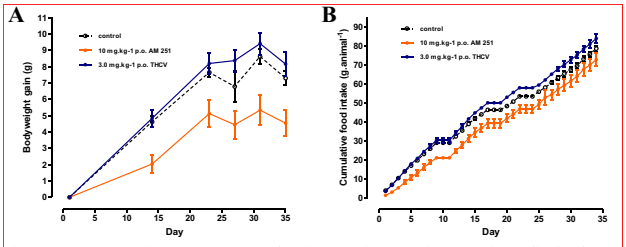

Figure 1. Body weight gain (A) and cumulative food intake (B) in obese mice dosed with THCV (3mg/kg) or AM251 (10mg/kg).

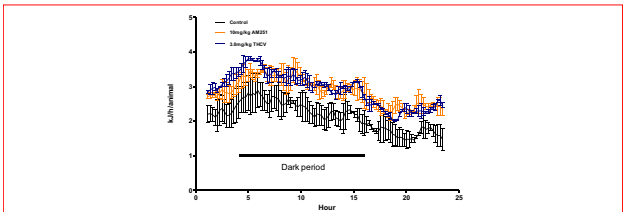

Figure 2. Twenty four hour energy expenditure in obese mice dosed with THCV (3mg/kg) or AM251 (10mg/kg).

Studies in *ob/ob* mice

THCV (3mg/kg daily for 28 days) had no chronic effect on food consumption or weight gain unlike the synthetic inverse agonist AM251 (Fig 1). However, it increased energy expenditure and the thermic effect of food (Fig 2). CBD (3mg/kg) produced significant reductions in plasma cholesterol whilst increasing HDL-cholesterol and reducing liver triglyceride content (Fig 3 and Fig 4).

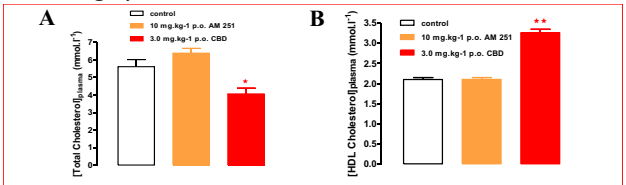

Figure 3. Total (A) and HDL cholesterol (B) in obese mice dosed with CBD (3mg/kg) or AM251 (10mg/kg).

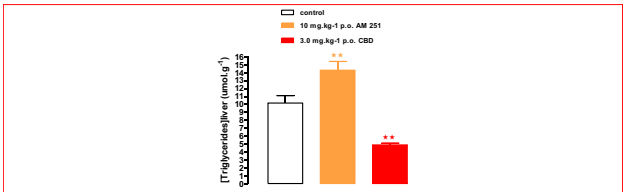

Figure 4. Liver triglycerides in obese mice dosed with CBD (3mg/kg) or AM251 (10mg/kg).

Studies in diet-induced obese mice

In a dose-response study, THCV (0.3-12.5mg/kg for 28 days) had no effect on food intake or body weight unlike AM251, but improved glucose tolerance, insulin sensitivity (HOMA) (Fig 5) and increased energy expenditure.

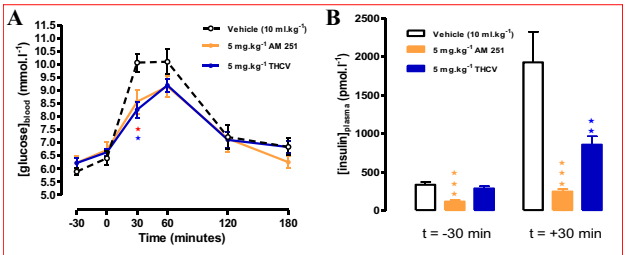

Figure 5. Glucose tolerance (A) and insulin levels post glucose challenge (B) in obese mice dosed with THCV (5mg/kg) or AM251 (5mg/kg).

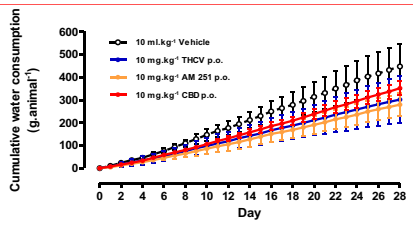

Figure 6. Cumulative water intake in diabetic mice dosed with THCV (10mg/kg), CBD (10mg/kg) or AM251 (10mg/kg).

Studies in *db/db* mice

Both THCV (10mg/kg) and CBD (10mg/kg) given for 28d produced small reductions in water intake (surrogate for glycaemic control) similar to AM251 (Fig 6). The plasma insulin concentration in control mice declined during the study as the diabetes progressed and this decline was attenuated by all three treatments (Fig 7). Morphometric analysis of the pancreas at the end of this study showed that THCV produced a significant islet cell protective effect (Fig 8).

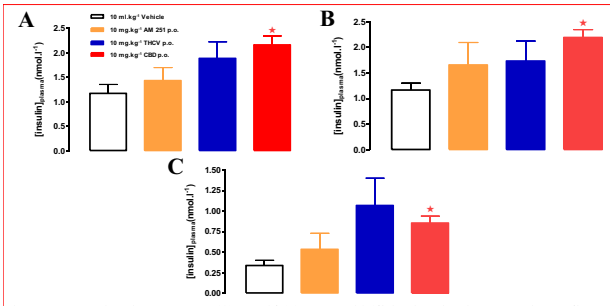

Figure 7. Plasma insulin levels day 7 (A), day 14 (B) and day 21 (C) in diabetic mice dosed with THCV (10mg/kg), CBD (10mg/kg) or AM251 (10mg/kg).

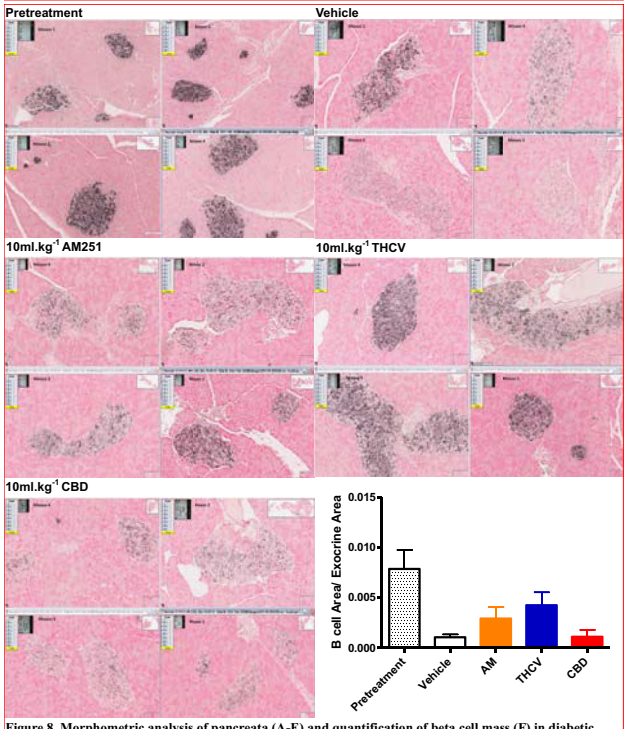

Figure 8. Morphometric analysis of pancreata (A-E) and quantification of beta cell mass (F) in diabetic mice dosed with THCV (10mg/kg), CBD (10mg/kg) or AM251 (10mg/kg).

Conclusions

- Non-psychoactive cannabinoids THCV and CBD have little or no effect on food intake, unlike AM251 and other inverse agonists.
- THCV increases energy expenditure and glucose tolerance.
- CBD lowers plasma cholesterol concentrations whilst increasing HDL-cholesterol.
- THCV, and to a lesser extent CBD, provide islet cell protection in diabetic *db/db* mice.