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Effect of *Centaurium erythraea* Rafn, *Artemisia herba-alba* Asso and *Trigonella foenum-graecum* L. on liver fat accumulation in C57BL/6J mice with high-fat diet-induced type 2 diabetes

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ABSTRACT

Ethnopharmacological relevance: *Centaurium erythraea* Rafn (CE), *Artemisia herba-alba* Asso (AHA) and *Trigonella foenum-graecum* L. (TFG) are traditionally used to treat type 2 diabetes in Algeria, previous studies have found that extracts of these plants were effective to treat or prevent experimental diabetes induced by high-fat diet (HFD).
Aim of the study: Describe the additional effects of these extracts on lipid tissue deposition in HFD.
Materials and methods: Male C57BL/6J mice were fed with HFD to induce type 2 Diabetes. Groups of mice were given plant extracts orally at 2 g/kg/bodyweight daily for 20 weeks during establishment of diabetes, or for 18 weeks after confirmation of diabetes at the 17th week. Liver and other tissue samples were stained with Oil Red O.
Results: Liver steatosis was confirmed with HFD. CE, AHA and TFG extracts improved liver steatosis by the end of the preventive (20 weeks) and curative periods (35 weeks). This was most marked for CE extract ($p < 0.05$), less so with TFG and AHA. No steatosis was found in other tissues.
Conclusion: CE extract had a clear hepatoprotective effect in this mouse model of diet-induced type 2 diabetes. AHA and TFG had a minimal or no significant effect on steatosis. Beyond its effect as an antidiabetic agent, CE may also be promising to prevent or treat non-alcoholic liver steatosis.

1. Introduction

In Algeria, a wide range of medicinal plants are used in folk medicine for the treatment of different disease and many are used for the treatment of diabetes such as *Centaurium erythraea* Rafn (CE) or Mraret lahnach, *Artemisia herba-alba* Asso (AHA) or Chih and *Trigonella foenum-graecum* L. (TFG) or Halba (Helba) as they are commonly named in Algeria (Hamza et al., 2010, 2011, 2012).

C. erythraea Rafn (CE) has been used in traditional medicine for its depurative, sedative, antipyretic and anti-inflammatory effects (Kumarasamy et al., 2003; Valentao et al., 2001, 2002). CE is also used in the treatment of diabetes (Hamza et al., 2010, 2011; Stefkov et al., 2014). *A. herba-alba* Asso (AHA) is also used for the treatment of diabetes, for its antihyperglycemic (Al-Khazraji et al., 1993; Al-Shamaony et al., 1994; Hamza et al., 2010; Mansi et al., 2007; Marrif et al., 1995; Stefkov et al., 2014; Testekin et al., 2007) and hypoglycemic effect (Hamza et al., 2011). *T. foenum-graecum* L. (TFG) has been traditionally used in the treatment of diabetes but most models on which TFG was tested were experimentally induced by streptozotocin or alloxan, which results in models closer to type 1 diabetes than the type 2 diabetes that is the most prevalent in human adults (Abdel-Barry et al., 1997; Ajabnoor and Tilmisany, 1988; Broca et al., 1999; Hannan et al., 2007; Khosla et

Abbreviations: HFD, High fat diet; TFG, *Trigonella foenum-graecum*; AHA, *Artemisia herba-alba* Asso; CE, *Centaurium erythraea* Rafn; HDL-cholesterol, High-density lipoprotein; HOMA, Homeostasis model assessment; STD, Standard diet; NASH, Non alcoholic fatty liver disease; i.p, Intraperitoneal administration; OR, Odds ratio; TG, Triglycerides; ADP, Adenosine 5′-diphosphate

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al., 1995; Mowla et al., 2009; Vats et al., 2003; Zia et al., 2001). We have tested TFG during development of high fat diet induced diabetes and in mice with established diabetes (Hamza et al., 2010, 2011). TFG has also been reported to have antioxidant properties (Anuradha and Ravikumar, 2001; Belguith-Hadriche et al., 2010; Dixit et al., 2005) and hypocholesterolaemic effect (Belguith-Hadriche et al., 2010; Hamza et al., 2012; Hannan et al., 2007; Valette et al., 1984).

In addition to the development of diabetes mellitus, high-fat diet results in dyslipidemia and tissue steatosis, especially potentially severe non-alcoholic liver steatosis. In addition, there is a link between the presence of type 2 diabetes and the severity of the liver injury (Hickman and Macdonald, 2007).

Non alcoholic fatty liver disease or steatohepatitis (NASH) is characterized by fat accumulation in the liver, which can progress to hepatic cirrhosis (Agro et al., 2009). The worldwide prevalence of non-alcoholic fatty liver disease continues to increase and corresponds to the frequency of the systemic complex known as metabolic syndrome (Farrell and Larter, 2006). NASH is increasingly recognized as the hepatic manifestation of insulin resistance and metabolic syndrome (Marchesini et al., 2001, 2003). Dixon et al. (2001) showed that insulin resistance and systemic hypertension, features of the metabolic syndrome, are independently associated with advanced forms of NASH. At present, no efficient remedy for NASH exists. Treatment is still centered on lifestyle changes, weight loss, healthy eating and exercise (Caldwell and Lazo, 2009). There are no reports on the effects of herbal therapies on experimental NASH.

In a previous study we found that extracts of *C. erythraea* Rafn (CE), *A. herba-alba* Asso (AHA) and *T. foenum-graecum* L. (TFG) could prevent and oppose the development of high fat diet-induced diabetes in C57BL/6J mice (Hamza et al., 2010, 2011, 2012). We report here the effects of diet and treatment on liver and tissue histology and lipid deposits in these HFD diabetic mice.

2. Materials and methods

2.1. Plant extracts

The air-dried parts of plants were botanically authenticated, and voucher specimens (NH-H 21, *C. erythraea*; NH-H 07, *A. herba-alba*; NH-H10, *T. foenum-graecum*) have been deposited in the Nutrition Department (Constantine 1 University, Algeria).

Hydroethanolic plant extracts of *C. erythraea* Rafn (CE), *A. herba-alba* Asso (AHA) and *T. foenum-graecum* L. (TFG) were prepared as previously reported (Hamza et al., 2010, 2011, 2012).

In brief, the dried aerial parts of CE, AHA and dried seeds of TFG were homogenized to a fine powder. Extracts were prepared as follows: the powdered plants were soaked in water–ethanol (2/8, v-v) solution (1:5, plant weight:solvent volume) for 24 h, then the solution was filtered, solvent replaced and the maceration renewed for another 24 h. After filtration, the filtrate was concentrated under reduced pressure at 50 °C and then freeze-dried.

2.2. Study design

Ten groups of 10 male C57BL/6J mice (4 weeks old at the start of the study) were studied, five for the preventive treatment study (weeks 0–20), and five for the curative study (weeks 17–35). In each part, a control groups was fed standard diet (STD); and four groups of animals were fed a high fat diet (HFD): one group was fed HFD alone and the three other groups were fed HFD and treated daily with plant extracts CE, AHA and TFG at the dose of 2 g/kg body-weight distilled in water *via* intragastric intubation. Extracts were administered from the beginning of the study to week 20 in the preventive study and from the 17th week to the 35th week of study in the curative group (Hamza et al., 2010, 2011, 2012).

Principles of laboratory animal care (Guide for the Care and Use of Laboratory Animals), published by the US National Institutes of Health (NIH Publication 85-23 revised 1996) were followed.

2.3. Biological sample collection and surgical procedure

After 4 h of fasting, the mice were anaesthetized using pentobarbitone (100 μl/100 mg i.p.) repeated if necessary and then sacrificed by cardiac puncture. Immediately after death, the abdomen was opened by a midline incision and the liver, kidney and muscle were quickly removed, harvested and weighed. Livers, kidney and skeletal muscle were then prepared for histological examination.

2.4. Histological examination of mice liver, kidney and skeletal muscle

Livers were weighed and dissected. Liver samples from different lobes were assembled, coded and sections embedded in the OCT medium (medium for frozen section), and immediately flash frozen in isopentane cooled in liquid nitrogen. They were then stored at −80 °C until further use. Organs were cut into 5-μm thick sections (Leica CM1850 UV microtome), mounted on glass slides and stained with Oil red O. The sections were counter-stained with Mayer's hematoxylin. Steatosis was analyzed by light microscopy.

The kidneys were rapidly excised longitudinally. One longitudinal section was prepared as above. One fragment of the right quadriceps muscle, of about 10×5 mm^2 in size was immediately placed in liquid nitrogen and then stored at −80 °C until further use.

Each stained liver, kidney and muscle section was analyzed for the severity of hepatocytes, kidney and intramyocellular fat accumulation. The extent of hepatocyte lipid accumulation was then scored based on the percentage of hepatocytes that contained macrovesicular fat.

Macrovesicular steatosis was graded 0–3 based on percent of hepatocytes in the biopsy involved (grade 0: none or absent; 1: less than 33%; 2: 33–66%; 3: more than 66%) (Kleiner et al., 2005); zonal distribution of steatosis and the presence of macrovesicular or microvesicular steatosis were noted. The pathological changes were evaluated and photographed using an Eclipse 50i Nikon optical microscope controlled by the software Nickon-NiS-elements D.

Histological evaluation included a semi-quantitative and quantitative analysis of the presence of micro and macrovesicular fat. All sections were coded and analyzed blindly by the pathologist without knowledge of related characteristics or diet (semi-quantitative analysis). The area of Oil red O-stained fatty droplets to the total tissue area was calculated (quantitative analysis) by using the analysis software ImageJ (ImageJ, US National Institutes of Health, Bethesda, Maryland, USA).

The results of the histological analysis were confronted to the biochemical results (blood glucose, plasma triglyceride, total cholesterol, HDL-cholesterol, insulin and HOMA) obtained in the same animals in our previous studies (Hamza et al., 2010, 2011, 2012).

2.5. Statistical analysis

Data analysis was conducted after database lock using SAS® software (SAS Institute, Version 9.4, North Carolina, USA). Descriptive variables are presented as mean and standard deviation (SD). Comparison between mice groups were performed using Kruskal–Wallis test. The significant multiple comparisons tests between each