Phytomedicine 19 (2012) 846–853

Contents lists available at SciVerse ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Cimicifuga racemosa and its triterpene-saponins prevent the Metabolic Syndrome and deterioration of cartilage in the knee joint of ovariectomized rats by similar mechanisms

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ARTICLE INFO

Keywords:
Ovariectomized rats
Metabolic Syndrome
Fat tissue
Cholesterol
Joint cartilage
Cimicifuga racemosa

ABSTRACT

Purpose: An unphysiologic accumulation of fat cells in many parts of the body including abdomen and joints results in increased production of pro-inflammatory cytokines which have adverse effects on serum lipids, glucose and on joint cartilage. The special extract of *Cimicifuga racemosa* CR BNO 1055 was shown to reduce the size of the abdominal fat depot. It was therefore tempting to test whether this extract, its saponin and its unpolar and polar fractions S- and R-fraction respectively (no quotation) also reduce fat depots and fat cell accumulation in a fat depot located in the lower hind leg (called paratibial fat depot = PFD), in joint fat pads (in the knee joint this is called Hoffa's fat pad) that occur in response to ovariectomy and whether this was accompanied by reduced serum lipids, glucose and improved cartilage features in the knee joint.
Methods: Rats ($n = 10$/group) were ovariectomized (ovx) and fed with CR BNO 1055, S- or R-fraction containing food (average intake 8.2, or 2.05 or 7.07 mg/day/animal) for 4 weeks. Ovx rats kept under no additive-containing food served as controls. The sizes of the PFD, of Hoffa's fat pad and of the cartilage thickness of the knee joints were determined by quantitative computer tomography and histomorphometrically. In the serum cholesterol, leptin and glucose levels were measured.
Results: High load with fat tissue in the PFD and in the knee joints was present in the ovx rats. Treatment with CR BNO 1055 and its S-fraction reduced fat load of both, Hoffa's fat pad and of the PFD significantly and this resulted in reduced body weight which was significant under CR BNO 1055. Fat load in the PFD correlated significantly with the height of serum leptin and cholesterol. The fat load in the knee joint correlated inversely with the size of knee cartilage tissue.
Conclusions: High fat load of the body increases following ovx and this causes increased serum leptin, cholesterol and glucose levels. Following ovx the size of Hoffa's fat pad increases also significantly and this has adverse effects on knee cartilage tissue. Therefore, increased fat tissue in joints appears to belong to the Metabolic Syndrome. This effect can be largely prevented by CR BNO 1005 and its S- but not by its R-fraction. Hence, the saponins in CR BNO 1055 may be useful in preventing the Metabolic Syndrome and osteoarthritis.

Introduction

It is now well accepted that two types of obesities do exist. The pear or gynoid type of fat distribution stands for accumulation of fat tissue in the hip and thigh region. In case of the apple or android type of obesity, fat accumulates in the abdominal cavity. This visceral fat has negative features; it secretes pro-inflammatory cytokines and adipokines such as TNFα, IL6, adiponectin, leptin and others (de Ferranti and Mozaffarian 2008; Klein-Wieringa et al. 2011; Rai and Sandell 2011) which are responsible for the development of hypercholesterolemia, arteriosclerosis, heart attacks and type II diabetes. These diseases are nowadays summarized as the Metabolic Syndrome (Ford et al. 2002; Watts and Karpe 2011).

Rheumatoid osteoarthritis is a common disease which is due to autoimmune inflammatory processes (Hazes and Luime 2011). Another type of arthritis is the formerly called arthrosis which

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0944-7113/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2012.03.001

occurs primarily in hip and knee joints. Since it develops primarily in overweight persons it was assumed that the overweight is the exclusive reason for the deterioration of cartilage in the knee (Bliddal et al. 2011). Lately evidence accumulates that fat pads, located in joints, also pay a role in the generation of arthrosis (Gandhi et al. 2011; Klein-Wieringa et al. 2011; Rai and Sandell 2011). These fat pads, particularly, Hoffa's fat pad in the knee joint are often enlarged in overweight persons (Hazes and Luime 2011). The adipocytes in joint fat pads share the negative features of visceral fat cells by secreting pro-inflammatory adipo- and cytokines and cause, therefore, local inflammatory processes in the joints. The secreted pro-inflammatory cytokines inhibit maturation of chondrocytes and cause the increased formation of matrix metalloproteinases which destroy the infrastructure of joint cartilage tissue (Rai and Sandell 2011). In the knee and hip joints these effects are augmented by the overweight of the obese people. Hence, arthrosis is not only a degenerative disease due to mechanical compression of cartilage tissue, but an inflammatory component is at least as important in the development and progression of this disease. Because of these inflammatory processes which also occur in the bone, arthrosis is now called osteoarthritis which in many patients is an integral part of the Metabolic Syndrome.

Ovariectomized (ovx) rats become obese and develop a Metabolic Syndrome including hyperglycemia (Chen and Heiman 2001; Gorres et al. 2011; Seidlova-Wuttke et al. 2003; Zoth et al. 2010). Furthermore, we demonstrated recently that ovx has adverse effects on joint cartilage tissue by thinning of the cartilage layer. This is suggestive to us that the ovx rat is a good model for the study of the Metabolic Syndrome and of osteoarthritis (Kapur et al. 2010).

In several reports we gave evidence that the special extract of *Cimicifuga racemosa* (CR) BNO 1055 prevents visceral obesity in ovx rats (Seidlova-Wuttke et al. 2003). Therefore, it was tempting to study the possibility that this extract can prevent ovx induced hyperlipidemia and hyperglycemia. Furthermore we tested whether CR-BNO 1055 resulted in less articular fat accumulation in the knee joint and whether this correlated with the size of the cartilage tissue in this joint.

In addition to the special extract CR BNO 1055 we also tested the saponin fraction (S-fraction) and a fraction containing the more polar substances of this extract (R-fraction). Thus, we tried to solve the question which of these fractions was responsible for the obesity preventive effects and the possibly accompanying reduction of hyperlipidemia and whether the fraction that prevented development of obesity was also effective to ameliorate an increased size of Hoffa's fat pad and thereby the adverse effects of ovx on cartilage tissue in the knee joint.

Materials and methods

Allowance to perform the animal experiments was obtained from the district authorities of Braunschweig, Germany (permission no. Az. 33.425002-082/06). Three-months-old female SD-rats (Winkelmann, Borken, Germany) were adjusted to the animal facilities of the Göttingen University Hospital and kept in groups of six in Makrolon cages (type 4) under a 12-h light, 12-h dark cycle at room temperature of 24–26 °C and relative humidity of 50–55% with food and water ad libitum. After 4 weeks adjustment to the animal facilities, the rats weighing 270 ± 4.4 g were ovx and groups randomized according to the bodyweights (BW) such that each group had identical BW. They were fed with soy-free food (V 1355 R-Z, 10 mm, poor phytoestrogens, ssniff, Soest, Germany) in order to eliminate exposure to soy-derived estrogenic compounds found in regular rodent show. Isocaloric protein supplementation was secured by added potato proteins. The test substances were the special CR extract CR BNO 1055 and the thereof fractionated lipophilic and hydrophilic compounds. All test extracts were produced by Bionorica SE (Neumarkt, Germany) as follows:

Plant material

As plant material the special extract from *Cimicifuga racemosa* CR BNO 1055 (Bionorica SE, Neumarkt, Germany) was used, of which the preparation was described previously (Kapur et al. 2010). This extract stems from rhizomes of *Cimicifuga racemosa* which was grown, harvested and extracted under rigidly controlled conditions.

Chemical and reagents

For the fractionation process we used dichloromethane (Emsure®, Merck, Darmstadt, Germany). Purified water for the separation process and as solvent for the HPLC system was produced by the Milli-Q Water Purification System (Millipore GmbH, Schwalbach, Germany). Analytical grade ethyl acetate and formic acid for TLC analysis were purchased from VWR International GmbH (Darmstadt, Germany). The eluents for the chromatographic analysis, acetonitrile and methanol, were gradient grade (Merck, Darmstadt, Germany). Pure formic acid 99–100% as additive for the mobile phase was purchased from VWR.

Fractionation process

The separation of the triterpeneglycosides from the hydrophilic components of the special extract BNO 1055 was obtained via liquid–liquid-extraction (LLE). The crude extract was partially dissolved in water and sonicated for 15 min. For improved separation of the phenylpropanoids the pH-value of the suspension was adjusted to 8.5 and dichloromethane was added and mixed for 1 h at 33 °C. The mixture was transferred into a separatory funnel to separate the lipophilic from the hydrophilic phase. After partition of the dichloromethane phase the LLE was repeated.

In order to obtain a solvent-free dichloromethane fraction, the pooled fractions were evaporated by using a rotary evaporator (Büchi Labortechnik GmbH, Essen, Germany) and lyophilized. The quality of separation of the obtained fractions was analyzed by TLC, HPLC-ELSD and HPLC–MS.

Sample preparation

For TLC and HPLC-ELSD analysis the native dry extract and the dried fractions were dissolved in 100% methanol (10 g/l) and sonicated for 15 min at room temperature. For HPLC–MS samples were prepared similarly with a final concentration of 2.5 g/l. Prior to analysis all samples were filtered through a Chromafil® PET-45/25 filter (Macherey-Nagel GmbH & Co. KG, Düren, Germany).

Instrumentation and chromatographic conditions

TLC analysis was performed with Automatic TLC Sampler 4 (Camag, Muttenz, Switzerland) using winCATS 1.4.6 software (Camag, Muttenz, Switzerland). Separation was achieved with a HPTLC silica gel 60 F254 20 cm × 10 cm plate (Merck, Darmstadt, Germany). Mobile phase consisted of ethylacetate:formic acid:water (95:5:5). The application volume of each sample was 10 μl, sprayed in 15 mm bands. Detection of the zones was achieved by immerse in 10% sulfuric acid and following heating for 5 min at 105 °C. Visualization of the fluorescence at 366 nm was assessed by Desaga ProViDoc® Version 5.4. (Sarstedt, Nürnbrecht, Germany) equipped with a Canon Powershot G9 camera.