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ORIGINAL ARTICLE

Antioxidant and anti-inflammatory effects of *Marrubium alysson* extracts in high cholesterol-fed rabbits

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Abstract The antioxidant and anti-inflammatory effects of hexane (HEXA), chloroform (CHLORO), ethyl acetate (EA) and total alcoholic (T. ALCOH) extracts of *Marrubium alysson* in hypercholesterolemic-fed rabbits were evaluated. Hypercholesterolemia was induced in male rabbits by high cholesterol diet (HCD) (350 mg/kg) for 8 weeks. Hypercholesterolemic rabbits were allocated into groups, treated with simvastatin (SIM 5 mg/kg), different extracts of *M. alysson* at two doses of 250, 500 mg/kg. A normal control group and an HCD control one were used for comparison. Lipid profile, as well as oxidized low density lipoprotein-cholesterol (ox-LDL-C), myeloperoxidase activity (MPO) and superoxide anion production ($O_2^{\cdot -}$), C-reactive protein (CRP) and monocyte chemoattractant protein-1 (MCP-1) were also evaluated. In addition, histological examination of ascending aorta was performed. We found dyslipidemia associated with significant increases in ox-LDL-C 123.5 ± 9.8 nmol MDA/mg non-HDL, MPO activity 0.08 ± 0.05 U/100 mg tissue and $O_2^{\cdot -}$ production 3.5 ± 0.3 nmol cytochrome C reduced/min/g tissue × 10^{-4} in

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hypercholerterolemic rabbits. In addition, there was a significant increase in CRP 6.6 ± 0.49 μmol/L and MCP-1 190.9 ± 6.4 pg/ml and its mRNA expression in HCD. Intima appeared thick with thick plaques surrounding the intima and luminal narrowing. SIM, EA and HEXA extracts of *M. alysson* had lipid lowering effect, decrease in ox-LDL-C, MPO, $O_2^{\cdot -}$, CRP and MCP-1 mRNA expression with improvement of the pathological picture. *M. alysson* enhanced the stability of plaque, had lipid lowering, anti-inflammatory and antioxidant activities.

1. Introduction

Marrubium alysson (*M. alysson*) is an asterid dicot genus of Old World aromatic herbs; includes horehound, a genus of about 40 species of flowering plants in the family Lamiaceae. It has been used in traditional medicine for various purposes. It was chosen in our study for its wide distribution in Egypt; it is reported to have a hypoglycemic effect and influence on bile secretion (Blumenthal et al., 2000). It is used also in the treatment of cold, cough and asthma (Louhaichi et al., 2011), as a diuretic (Caceres et al., 1999), appetizer, astringent, gastroprotective (Paula et al., 2011) and antiviral (Edziri et al., 2011).

High cholesterol diet and oxidative stress increase serum total cholesterol (TC) and LDL-C levels (Jeon et al., 2001). Oxidative modification of LDL-C plays a major role in the pathogenesis of atherosclerosis. The first stage of atherogenesis is characterized by an influx and accumulation of LDL-C in the intima, followed by recruitment of blood-derived monocytes and lymphocytes to the developing lesion (Steinberg, 2005). Subsequently, LDL-C is oxidized by free radicals; ox-LDL-C induces a multitude of cellular responses which lead to vascular dysfunction (Hulten et al., 2005).

MCP-1, a member of the C–C chemokine β subfamily, causes the recruitment of monocytes, and as such may contribute to the initiation and maintenance of inflammatory reactions in the vascular tissues (Feng et al., 2005). Moreover, MCP-1 has broader roles in adipocyte physiology than inflammatory cell recruitment (Sartipy and Loskutoff, 2003). MCP-1 has a direct angiogenic effect on endothelial cells (Low et al., 2001), it contributes indirectly to inflammation by acting on the liver to produce acute phase proteins (Hug and Lodish, 2005). Among these acute phase proteins is CRP. In recent years, circulating levels of CRP have been clearly identified as a powerful independent risk factor for cardiovascular diseases (Ridker et al., 2000).

Since the involvement of free radicals in the pathophysiology of atherosclerosis was proposed, antioxidant supplementation arose as a potential strategy for the management of this disease (Hakimoğlu et al., 2007). The antioxidant activity is higher in medicinal plants, and because of their perceived effectiveness, with minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely (Miliauskas et al., 2004).

Our study aimed to investigate the potential benefits of total alcoholic (T. ALCOH), ethyl acetate (EA), chloroform (CHLORO), hexane (HEXA) extracts of *M. alysson* for their antioxidant and anti-inflammatory effects in hyper-cholesterolemic rabbits in order to verify the activities for which the plant is used in traditional medicine. Also, the toxicity of different extracts was evaluated.

2. Materials and methods

2.1. Plant collection and preparation of extract

Marrubium alysson was collected from Burg El-Arab at Alexandria during April to June 2010. The collected plant was identified by Dr. Saneia Kamal, Assistant Professor, Faculty of Science, Alexandria University. A voucher sample (*M.A.* – 1) was kept at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Egypt. The plant was air-dried, finely powdered (4 kg of the dry plant), then extracted. The cold maceration technique was used for extraction of the plant. The powdered plant was soaked in methanol at room temperature. After seven days, the extract was filtered under vacuum through Whatman filter paper No. 1. The residue was again dipped in methanol for an additional seven days and filtered thereafter. The filtrate was combined and methanol was evaporated under vacuum, using a rotary evaporator (Buchi Rotavapor R-200) at 55 °C to yield viscous greenish-colored extract. The quantity of the extract obtained from *M. alysson* was 300 g (15%).

2.2. Fractionation

Distilled water was added to the methanol solution in a ratio of 2:1, followed by successive fractionation with HEXA (3 × 200 ml), CHLORO (3 × 200 ml) and EA (3 × 200 ml). Each extract was concentrated separately using vacuum rotary evaporator and stored at 4 °C till use. Two doses of 250 and 500 mg/kg were selected and used in this study. The two doses were prepared by dissolving appropriate amount of these viscous extracts in 1 ml Tween 20. This was followed by adding 9 ml of 0.9% NaCl to each mixture. The vehicle was obtained by dissolving 1 ml of Tween 20 in 9 ml of 0.9% NaCl (Irshaid and Mansi, 2009).

2.3. Animals

Eighty-eight male New Zealand White rabbits (2–2.5 kg) were obtained from the Egyptian Organization for Biological Products and Vaccines. All the animals were housed in individual cages, left for 7 days prior to the study to acclimatize and received standard pellets (15% protein, 2.5% lipid, 15% cellulose, 14% clay, 13% water) (Sezer et al., 2011) during this time. The animals were maintained on normal light–dark schedule and temperature 25 ± 3 °C throughout the experiment and given free access to water. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the Faculty of Pharmacy, Suez Canal University (Ismailia, Egypt).