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ANTI-INFLAMMATORY EFFECT OF BERBERIS VULGARIS CRUDE EXTRACT IN VIVO

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Atherosclerosis is initiated by damage to the endothelium followed by accumulation of inflammation tissue, foam cell formation, migration and proliferation of vascular smooth muscle cell and rupture of the plaque causing formation of thrombus. The study aims to determine the anti-atherogenic or inflammatory effect of BVFCE and its underlying mechanism in in vivo model. Five groups of four rabbits were treated with normal diet (Group I), Berberis vulgaris fruit aqueous extract [25mg/kg(group II), 50mg/kg (group III) bodyweight], aspirin 30mg/kg bodyweight (group IV) and cholesterol 2% (group V) for 10 weeks of treatments. At the end of the study, the rabbits blood were collected and aorta harvested post euthanized. Serum of the blood tested for cytokines (interleukin- 6 and tumour necrosis factor – alpha while aortic plaque composition of intimal and medial cell population in aorta was assessed by macrophages specific antibody called monoclonal mouse antibody directed against RAM-11 using immunohistology-staining techniques. The fatty streak lesion were analysed macroscopically by Sudan IV and microscopically by using the Olympus Cell ^F Imaging software and served to compute intimal area. The total macrophage-rich (RAM-11-positive) area measured using a manual contrast-based, area analysis function of the Olympus Cell ^F Imaging software. Macrophage density calculated as the ratio between macrophage and intimal areas. The RAM-11– positive were cells identified by brown staining, were divided by the total number of intimal and medial cells. Macrophage infiltration into the aorta wall showed dose dependent inhibition RAM11-positive cells in the vessel wall from 1.1 ± 1.2% of total cells with Berberis vulgaris 25mg/kg to 0.58 ±1.8% with Berberis vulgaris extract (50mg/kg) while positive control showed 1.9 ± 1.1%. This study demonstrates that Berberis vulgaris fruit aqueous extract is associated with changes in rabbit aorta plaque composition that favor lesion stability, by a reduction in lipid content and inflammation, in this case, interleukin -6 and macrophages, which gave an effect of anti -inflammation.

Keywords: inflammatory response, RAM II, atherosclerosis

INTRODUCTION

Inflammation is a condition in response to tissue injury. It can be divided into acute and chronic phases. Acute phase is described by increased blood flow and vascular permeability along with the accumulation of fluid, leukocytes, and inflammatory mediators such as cytokines.

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Cytokines such as interleukin- 1(IL-1), interleukin -6 (IL-6), interleukin -8 (IL8), interleukin- 11(IL-11) andor necrosis factor- alpha (TNF- α) play important roles in mediating acute inflammatory reactions. [1]. Inflammation continues during atherosclerosis development and mediated released of more proinflammatory cytokines[2]. Study by Hotamisligil [3] had support the existence of inflammatory response involving the adipose tissue and its potential role in atherosclerosis.

Berberis Vulgaris (B. vulgaris) or known as "barberry" is a thorny plant with yellow flowers and small red fruits. It is a native herb to Europe and Asia, and can be found growing wildly from Canada to Pennsylvania. In traditional Chinese medicine, barberry was mentioned more than 3000 years ago to have diverse medicinal properties including antimicrobial, antiemetic, antipyretic and antipruritic. It has been used as treatment for cholelithiasis, jaundice, dysentery, leishmaniasis, malaria and gall stones [4]. Moreover, barberry may offer health care benefits as an anti hypertensive and vasodilator agent [5]. B. vulgaris extract has been shown to play a prominent role in promoting apoptosis in the treatment of hepatocarcinogenic rats [6]. Atherosclerosis is a disease initiated by an inflammatory state. In this study, an atherosclerosis - induced rabbits model were used to determine the potential anti-inflammatory/anti atherogenic properties of B. vulgaris fruit aqueous extract.

METHODS AND MATERIALS

Male New Zealand white rabbits weighted around 1-1.2 kg were cage for 12/12 hours light/dark cycle at 25±5∘C and food and water were given ad libitum for 7 days. The rabbits were divided into five groups and fed with normal pallet diet (group I) 2% cholesterol with different dosage of Berberis vulgraris fruit aqueous extract (BVFAE) (25mg/kg and 50mg/kg) (group II and III), 2 % cholesterol diet only (group IV) and 2% cholesterol with aspirin (10mg/kg)(group V) for 10 weeks in a row. At the end of the experiment), blood samples were taken from auricular vein and subjected for Tumor necrosis factor-alpha (TNF-α), and Interleukin -6 using standard commercial ELISA kit (Cloud-clone Corp, USA). The rabbits were euthanized by an intravenous injection of sodium pentobarbital (200mg/kg) and their aorta were dissected and subjected for macroscopic and microscopic examination of atherosclerotic plaques.

Dried B. vulgaris fruits were imported from certified herbal marketing company in Tehran, Iran. B. vulgaris prepared using decoction method of Movahedi et al [7] with slight modification. The fruits were further dried in an oven for 3 days at a constant temperature of 60∘C. The fruits were cut into small pieces and grounded into fine powder using a dry grinder. For each 100g of B. vulgaris powder, 4000mL of distilled water was used. The mixture of the powder and water was heated up 70∘C to reduce the water content to 1000mL through evaporation. After these phases, the residues were filtered using Whatman No.1 filter paper and the extract was chilled in bottles and kept in the chiller at 4∘C until being used.

Aortas were excised, fixed for 24 h in 4% paraformaldehyde, and embedded in paraffin. Slices (5 µm thick) were sectioned and subjected for H & E staining and immnunohistochemistry staining H&E staining technique used to stain the tissue by using Autostainer XL following method described by McManus and Mowry (1960). The slides underwent hydration, colorization and dehydration process as a standard procedure. After that, slides were mounted with cover slips using DPX gum. The slides were dried in fume hood in a room temperature and examined using light microscope at 40x, 100x and 200x magnification.

A macrophages specific antibody was used to evaluate the composition of intimal and medial cell population in aorta by immunostaining techniques. Macrophages were detected on adjacent slices by immunohistochemistry using a monoclonal mouse antibody directed against RAM-11, a marker of rabbit macrophage cytoplasm (dilution, 1:50) [8]. The luminal area and the area bounded by the internal elastic laminae were measured on each arterial cross-section using the Olympus Cell ^F Imaging software and served to compute intimal area. The total macrophage-rich (RAM-11-positive) area measured digitally using an automated, contrast-based, area analysis function of the Olympus Cell ^F Imaging. Macrophage density calculated as the ratio between macrophage and intimal areas.

ELISA The collected blood was centrifuged at 2600 g for 10 minutes. The serum obtained stored in – 80∘C prior to use. The serums were subjected for TNF-α and IL- 6 using ELISA kit. The data obtained were compared to a standard curve and plotted to get best-fit straight line and the equation used in determining the concentration of the sample.

Statistical Analysis

Data were expressed as mean ± SEM. Statistical differences between normal, treated, and control groups were determined using one-way repeated measures analysis of variance (ANOVA). Differences between groups were considered significantly different when *P* value was less than 0.05.

RESULTS AND DISCUSSIONS

B. vulgaris fruit aqueous extract (BVFAE) was tested on atherosclerosis induced rabbit models for inflammation properties. Oral treatment for 10 weeks together with 2% cholesterol diet with or without BVFAE treatments and aspirin as a positive control had been done.

As it shown in Figure 1, a significant lower levels of TNF- α was found in the normal group and aspirin treated group ($p<0.05$). No significant difference between BVFAE treated group however it showed better effects as compared to negative control. While, the significant lowest level of IL-6 was found in normal, aspirin and BVFAE treated group ($p<0.05$). The IL- 6 level in negative control group were significantly higher than normal and BVFAE group ($p<0.05$). BVFAE treated group showed significantly reduction of cytokines compared to negative control ($p>0.05$). The lesions were examining histologically and compared with those untreated animals using H&E staining shown in Figure 2.

The overall morphology of lesions was similar in BVFAE aspirin and control group with present of the fibrous areas, areas rich in foam cells and necrotic areas. However in concentration of 50mg/kg BVFAE, there is significant reduction in intima media ratio compare to control group ($p<0.05$) (data not shown).

Macrophages- derived foam cells were identified through immunohistochemical staining of sections for the macrophage marker RAM II (Figure 3). Macrophages were present both beneath overlying fibrous caps and at the surface of complex lesions from both treated and control animals.

This study has demonstrated the anti-inflammatory and also anti-atherosclerotic effect of BVFAE in a model of atheroclerosis induced rabbit. In this experiment, we used water decoction method to produce BVFAE to imitate regular usage in daily life.

The study showed that BVFAE reduce inflammatory mediator i.e. : IL-6 and TNF – α at 50mg/kg that indicate acute inflammation and reduction of macrophages per intimal area presenting for chronic inflammation process. These findings is in agreement with most of studies using *B. vulgaris* but using difference preparation extraction method[9-11].

There were several components in *B. vulgaris* that might be responsible for the anti inflammatory effect including tannins, saponins, flavonoids, alkaloids and steroids components [12]. There also reports thatsuggested berberine, the main compound in *B. vulgaris* inhibits the formation of foam cells by macrophages via enhancing liver X receptor - alpha - ATP binding cassette transporter A1

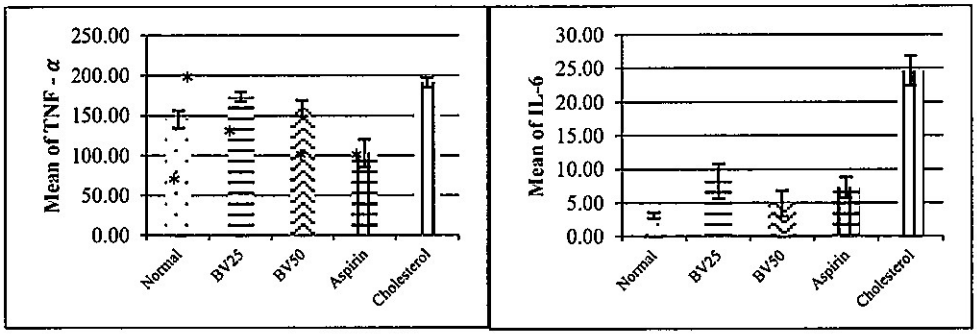

Figure 1: Effect of Different Treatment on Serum TNF-α and IL-6 of rabbits as compared to normal and control cholesterol group.Asterixes (*) means significantly different at $p<0.05$ based on one way ANOVA.Normal : Normal diet, BV25 : *B.vulgaris* 25mg/kg , BV50 : *B. vulgaris* 50mg/kg.

(LXRα- ABCA1)- dependent cholesterol efflux[13]. ABCA1 is a key factor in mediating cellular cholesterol efflux and act as reverse cholesterol transport with mediated via LXR nuclear hormone receptor. Besides, lysophosphatidylcholine (LPC) also had been reported to have potential of promoting cellular cholesterol effux. Both ABCA1 and LPC are potent chemotactic factors for monocytes that

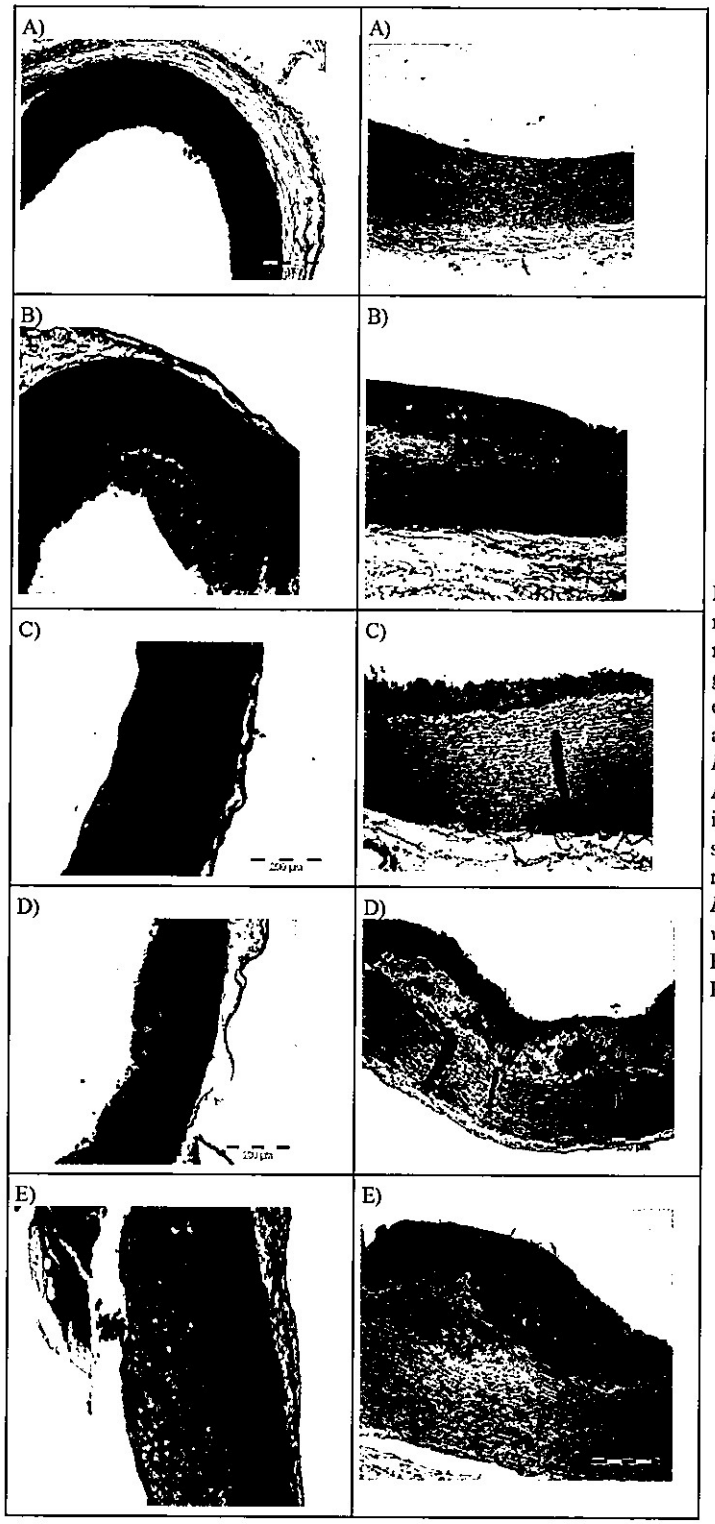

Figure 2: First column shows morphology of lesion from light microscopy of aorta in different groups. Second column represent examples of atherosclerotic lesions in atherosclerotic induced, treated with *B. vulgaris* and control rabbits groups; A-E. Macrophages (RAM-11 immunostaining) were seen mainly in subendothelial region of aorta. A) normal control rabbits B)25mg/kg *B.vulgaris* treated C) 50mg/kg *B. vulgaris* treated D) aspirin treated and E) cholesterol induced rabbit group. H&E, IHC x100

supported an anti inflammatory properties in present study [14]. Serum levels of various cytokines indicate inflammation-signaling pathways. In study on *B. vulgaris*, it had been reported that berberine, main compound present in this fruit reduced lipopolysaccharide- induced expression of inflammatory gene including inducible nitric oxide synthase (iNOS), cyclooxygenes-2 (COX-2) and IL-6 and the generation of nitric oxide and reactive oxygen species are the other pathways suggested for anti – inflammatory effects of B. vulgaris in atherosclerosis induced rabbit models. [15].

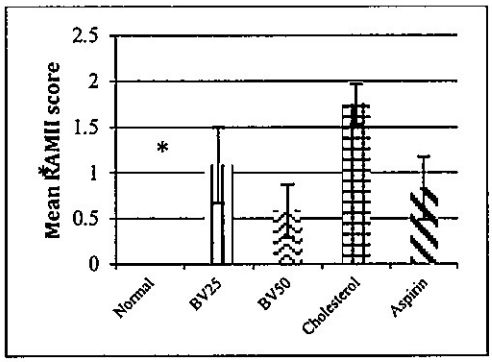

Figure 3: Mean RAM II Scoring of Aorta in Rabbit Treated with *Berberis vulgaris* with different concentrations as compared to Atherosclerosis non-treated control groups and aspirin as positive control group. Asterixes (*) means significantly different at $p<0.05$ based on one way ANOVA.Normal : Normal diet, BV25 : *B.vulgaris* 25mg/kg , BV50 : *B. vulgaris* 50mg/kg.

CONCLUSION

Evidences from this study contribute to the new finding towards anti inflammatory agent from a plant fruits, *B. vulgaris* contributing to the effort of treatment for cardiovascular disease The BVFAE play a prominent role in suppression of macrophages and cytokines upon treatment and it is dose dependent.

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