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MODULATORY EFFECTS OF *AGRIMONIA EUPATORIA* L. ON PROINFLAMMATORY CYTOKINES LEVELS IN EXPERIMENTAL MODEL OF METABOLIC DISTURBANCES

D. Ivanova[1], M. Nashar[1*], M. Radanova[1], K. Bratoeva[2], G. Bekyarova[2], Y. Kiselova[1]

[1]Department of Biochemistry, Molecular Medicine and Nutrigenomics,
[2]Department of Pathophysiology, Medical University Varna, Bulgaria

ABSTRACT

Great amount of scientific studies report that fructose intake can cause metabolic disturbances and liver inflammatory injury. This study was designed to evaluate the effect of aqueous-alcoholic extract of *Agrimonia eupatoria* L. (agrimony) on liver proinflammatory cytokines in fructose-fed rats for 12 weeks. Rats on fructose diet exhibited significantly higher levels of liver cytokines then the control rats on standard diet. Dietary supplementation with agrimony extract significantly decreased levels of hepatic C-reactive protein in fructose-fed rats as well as in animals on standard diet. Decreased levels of interleukin 6 were estimated in animals on fructose diet supplemented with agrimony but in controls the herb itself stimulated the production of this cytokine. Our findings demonstrated that agrimony had an immunomodulatory potential and could be considered in support to the folk medicine usage of the herb as remedy in inflammatory processes and for prevention of inflammation.

Key words: agrimony, chronic inflammation, fructose, rats, liver, interleukin 6, C-reactive protein, tumor necrosis factor alpha

INTRODUCTION

Chronic inflammation is considered as a pathogenic mechanism in metabolic and cardiovascular complications which often accompany obesity. The abnormal adipose tissue accumulation is the major factor for increased concentration of circulating biological markers of inflammation. The overproduction of tumor necrosis factor alpha (TNFα), interleukin 6 (IL-6) and C-reactive protein (CRP) is often involved in metabolic syndrome complications such as insulin resistance and cardiovascular diseases (CVD) (1). Along with hyperlipidemia and weight gain, the chronic liver injury is another pathogenic feature of obesity-related metabolic disturbances and the resident immune cells in liver may also contribute to maintenance of elevated proinflammatory cytokine levels (2-4).

Many studies have confirmed the potential of plant extracts and phytochemicals to modify obesity-related complications, particularly inflammatory responses (5).

Agrimonia eupatoria L. (agrimony) is a medicinal plant well-known from the folk medicine for its diverse beneficial effects, especially for its anti-inflammatory properties (6). Nowadays this herb is widely used without medical prescription as a remedy in various health complaints as well as in everyday life. The estimated antioxidant activity of agrimony strongly correlated with high polyphenol content of its extracts (7) and it was reported for cell cultures (8), as well as in experimental animals (9). Recently we have found that agrimony aqueous-alcoholic extract had the potential to improve markers of lipid metabolism in fructose-induced metabolic syndrome (10). The present study aimed to investigate the effect of agrimony aqueous-alcoholic extract on markers of inflammation in the same model.

MATERIALS AND METHODS

Plant material

The plant tested is a commercial product of Selibum Ltd., Varna, Bulgaria standardized according to the European Pharmacopoeia. The

***Correspondence to**: *Milka Nashar, Medical University-Varna, 55 Marin Drinov str. 9002 Varna, Bulgaria; nacharbg@yahoo.com*

technology of the commercial preparation of the dried plant product includes botanical identification prior to plant processing.

Preparation of 40% aqueous-ethanol plant extract

The dried and grinded plant material (150mg) was processed by triple extraction with 40% ethanol dissolved in distilled water (v/v), in proportion 1 g plant material per 20 ml solvent. The supernatants from all extraction steps were merged and dried using rotary evaporation system. The residue was dissolved either in distilled water - for treatment of standard diet-fed group receiving the agrimony extract (StD+A) or in 12.5% fructose - for treatment of fructose-fed group receiving the agrimony extract (FR+A).

Experimental animal procedure

The experimental protocol was approved by the Home Office for Care and Use of Laboratory Animals and performed according the ethical rules of the International Guiding Principles for Animal Research approved in Bulgaria. Male Wistar rats (b. w. 140-180 g) were housed at standard laboratory conditions with 12-h light/dark cycle and room temperature (20±2°C). The animals received water *ad libitum* and standard diet with the following composition: starch 50%, protein 20%, fat 4,5%, cellulose 5%, standard vitamins and minerals mix. After two weeks of adaptation period the animals were divided randomly into four treatment groups (n=6) as follows: a standard diet-fed group (StD), a fructose-fed group (FR) receiving 12.5% fructose corn syrup in the drinking water, a fructose-fed group treated with agrimony extract (FR+A) and a standard diet-fed group treated with agrimony extract (StD+A). After 12 weeks treatment period the animals were sacrificed with overdose of thiopental and livers were collected and homogenized for analysis.

Quantification of proinflammatory biomarkers

Concentrations of IL-6, TNFα (IBL International) and CRP (Helica biosystems, INC.) were measured in liver homogenates using commercially available ELISA kits.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by one-sample t-test (95% confidence interval) using GraphPadPrism v.5.00 software. Results with p values<0.05 were considered to be statistically significant. Data are presented as mean±standard error of mean (SEM).

RESULTS

Effect of Agrimonia eupatoria on IL-6 in liver

Liver concentration of IL-6 in fructose fed animals increased significantly as compared to the group on standard diet ($p<0.05$). The concentrations measured were 49.1±4.2 ng/g tissue for FR, and 33.4±2.6 for StD group, respectively. Although that the differences between FR and FR+A groups were not statistically significant, a tendency for a decrease of the cytokine levels was observed in the latter. Surprisingly, the supplementation with agrimony extract in healthy rats (StD+A group) led to an increase of the IL-6 levels after 12 weeks treatment period ($p<0.05$, versus StD group). The quantification of IL-6 levels is visualized in **figure 1A.**

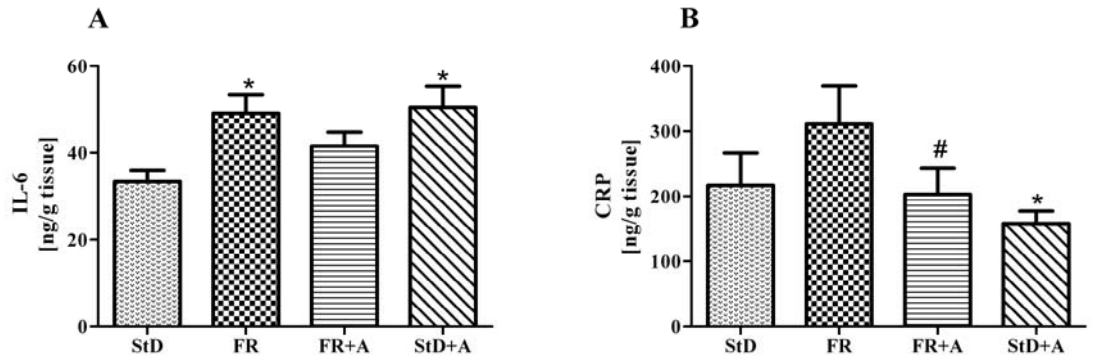

Figure 1. Concentration of IL-6 (A) and CRP (B) in rat liver after 12 weeks treatment period. Data are presented as mean±SEM; *p<0.05 vs. StD, #p<0.05 vs. FR.

Effect of Agrimonia eupatoria on CRP levels in liver

The 12 weeks of high-fructose diet induced slight elevation in the liver levels of CRP, as compared to rats fed with standard diet. However, the concentrations measured did not differ significantly between the two groups. Significantly lowered levels of CRP were estimated both groups supplemented with agrimony extract – the concentration measured for FR+A group was 202.7±40.6 ng/g tissue, versus 310.9±58.9 ng/g tissue for the FR group ($p<0.05$). Significant differences were detected in cytokine concentrations between StD+A and StD groups (158.1±19.2 ng/g tissue and 216.8±49.6 ng/g tissue respectively, $p<0.05$). Results are presented on **figure 1B**.

Effect of Agrimonia eupatoria on TNFα levels in liver

The levels of liver TNFα remained unaltered by fructose treatment, as well as by agrimony extract supplementation. The measured values were as follows: 14.7±0.8 (StD), 14.8±1.2 (FR), 13.5±1.4 (FR+A) and 12.3±1.1 (StD+A).

DISCUSSION

In the past three decades fructose has been used as a major sweetener by food industry and as a replacement of sucrose in the diet of subjects with diabetes. However, increasing body of evidence suggests that fructose induces non-alcoholic fatty liver disease (NAFLD) (11), accompanied by liver fat accummulation and inflammation. NAFAD is also the most common hepatic manifestation of obesity. The chronic inflammation observed in obesity has been related to the development of metabolic syndrome and diabetes type 2 and an increase in inflammatory cytokines such as TNFα, IL-6, monocyte chemotactic protein (MCP)-1, and IL-1 is reported (1). These cytokines and chemokines in obese subjects correlate with CRP levels, a marker of systemic inflammation. In accordance with this we have demonstrated that fructose induced an increase in liver IL-6 and CRP in fructose-fed animals (**Fig. 1A and B**).

Treatment of low-grade inflammation with conventional medicines could be avoided and replaced by herbal preparations with anti-inflammatory potential. The polyphenolic profile of *A. eupatoria* aqueous-alcoholic extract includes compounds such as procyanidins, kaempferol and quercetin glycosides that are recognized to possess anti-inflammatory properties (12). In our experimental settings supplementation with agrimony extract of fructose-fed animals resulted with a decrease in hepatic IL-6 and CRP levels in agreement with the scientific reports and traditional usage. Furthermore, we have previously demonstrated that agrimony successfully reduced hyperlipidemic markers and body weight in rat experimental model of metabolic syndrome (10).

In support to the anti-inflammatory activity of agrimony, treatment with the herbal extract of the animals on standard diet decreased CRP levels (**Fig. 1B**).

Surprisingly, healthy rats' supplementation with agrimony extract (StD+A group) led to an increase in IL-6 levels after 12 weeks of treatment period (**Fig. 1A**). There are controversial reports about the effects of various plant extracts on IL-6 levels. Along with the reports about the inhibitory effect, other authors report a stimulatory activity (13, 14). These data could indicate a possible immunomodulatory activity of the respective herb. Our previous findings demonstrate that *A. eupatoria* extract enhances also IL-6 gene expression in adipose tissue of rats on standard diet (15). This, together with the established inhibitory effect of the extract on CRP (**Fig. 1B**), allow us to conclude that *A. eupatoria* has an immunomodulatory activity in an experimental model of fructose-induced metabolic disturbances. Thus the anti-inflammatory potential of aqueous-alcoholic extract of *A. eupatoria* was for the first time demonstrated in vivo.

In this respect agrimony could be considered as a potential remedy for the treatment of low-grade inflammation and the related diseases, and also may be used for prevention of inflammation as a supplement to the everyday diet.

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