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Original Article

The influence of Atherophyton on the development of low-grade inflammation in experimental metabolic syndrome in Syrian golden hamsters

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Abstract

The study aimed to investigate the effect of the dietary supplement (DS) Atherophyton on the development of low-grade inflammation by assessing the effect of such cytokines against the background of experimental metabolic syndrome in Syrian golden hamsters. The experimental metabolic syndrome (EMS) in Syrian golden hamsters was induced by keeping the animals for 24 days on a diet enriched with fructose (60% of the total daily diet with complete replacement of drinking with a 10% fructose solution). According to our experimental study, in the conditions of EMS, the leading role in the pathogenesis of which is associated with the development of oxidative stress and subsequent activation of chronic subcellular inflammation, the use of Atherophyton contributed to the suppression of low-grade inflammation, as indicated by a decrease in cytokine levels: IL-1β, IL-6, TNF-α by 1.5 ($p<0.05$), 1.8 ($p<0.05$) and 1.7 ($p<0.05$) times, respectively, which in turn is due to the ability of biologically active substances of medicinal plant materials included in Atherophyton to affect the etiopathogenetic component EMS development and, accordingly, the chronic sub-inflammatory process. DS Atherophyton suppresses the development of low-grade inflammation against the background of EMS in Syrian golden hamsters.

Keywords: phytotherapy, cryotechnology, experimental metabolic syndrome, low-grade inflammation, atherophyton.

Introduction

Metabolic syndrome (MS) is a set of pathogenetically interrelated metabolic, hemodynamic, and hormonal disorders based on insulin resistance. Clustering of metabolic abnormalities is closely related to oxidative stress and inflammation [1]. Today, chronic inflammation and neurohumoral activation are considered mechanisms of insulin resistance. They are important components of the progression of MS and its subsequent transition to cardiovascular disease and type 2 diabetes [1, 2]. Excessive nutrition activates several pro-inflammatory signaling pathways, which leads to chronic low-grade inflammation in certain tissues and organs, affecting their proper function [3]. Adipose tissue, liver, muscles, and pancreas are themselves foci of inflammation in the presence of obesity [4]. In these tissues, macrophages and other immune cells infiltrate, associated with a change in the cellular population from an anti-inflammatory to a pro-inflammatory profile. These cells are crucial for producing pro-inflammatory cytokines, which act in an autocrine and paracrine manner, interfering with the transmission of insulin signals in peripheral tissues or inducing

β-cell dysfunction and subsequent insulin deficiency. Chronic low-grade inflammation, which underlies the pathogenesis of MS, is indicated by an increase in inflammatory markers such as IL-1β, IL-6, C-reactive protein, and TNF-α, observed in individuals with MS [5].

Given that low-grade chronic inflammation is increasingly understood as the basis of several pathological conditions [6], the ability to pharmacocorrect it is a pharmacodynamically advantageous component of potential pharmacological agents proposed for use in the pharmacotherapy of MS.

A dietary supplement (DS) Atherophyton produced by the Research and Production Pharmaceutical Company (RPPhC) AIM is a phytomedicinal product with a complex composition based on medicinal plant material (MPM) obtained by an innovative method of cryo-technological processing [7]. Atherophyton tablets are produced using cryogenic grinding technology [8]. Until recently, the lack of traditional methods of obtaining biologically active substances (BAS) from plant material did not allow for the production of truly effective drugs. The cryo-grinding technology eliminates all the disadvantages of traditional methods of obtaining biologically active substances and is now recognized as the most effective of all existing methods. According to the recommendations for using the DS Atherophyton, it can be used in the diet as a source of dietary supplements that help normalize blood cholesterol levels and improve the condition of the vessel walls [9]. Given that dyslipidemia is one of the determinants of metabolic syndrome and is pathogenetically associated with developing subchronic inflammation, we chose Atherophyton to study its effectiveness in metabolic syndrome.

The study aimed to investigate the effect of DS Atherophyton on the development of low-grade inflammation in experimental metabolic syndrome in Syrian golden hamsters.

Material and methods

The study was conducted at the Educational and Research Institute of Applied Pharmacy of the National University of Pharmacy (NUPh) (Kharkiv, Ukraine), certified by the State Expert Center of the Ministry of Health of Ukraine. During the experiment, the animals were kept in standard vivarium conditions with natural day-night light and free access to water and food. All manipulations were carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Treaty Series No. 123: Text amended according to the provisions of the Protocol (ETS No. 170), 2005) [10]. The NUPh Bioethics Commission approved the experiment (Protocol of March 25, 2021, No. 5).

Study design and experimental animals

The experiment used 30 male Syrian golden hamsters aged 12 weeks and weighing 150-185 grams, 6 animals in each experimental group.

The experimental metabolic syndrome (EMS) in Syrian golden hamsters was induced by keeping the animals for 24 days on a diet enriched with fructose (60% of the total daily diet with complete replacement of drinking with a 10% fructose solution) [11].

Animals were divided into 5 experimental groups: I normal (intact) control - hamsters that consumed a standard pelleted diet balanced in terms of proteins, fats, carbohydrates, essential trace elements and vitamins (manufactured by PF VITA, Obukhiv, Ukraine); Group II experimental metabolic syndrome (EMS) - hamsters that received a diet 60% enriched with fructose for 24 days [11]; Group III - hamsters that received intragastric (i.g.) Atherophytone tablets at a dose of 255 mg/kg during the last 10 days of EMS modelling [12]; Group IV - hamsters that received i.g. metformin (Siofor, 500 mg tablet, No. 60, manufactured by Berlinhemi, Germany) at a dose of 60 mg/kg during the last 10 days of EMS modelling [12]; Group V - hamsters that during the last 10 days of EMS modelling received of Arfazetin (Arfazetin, 1.5 g sachets, PJSC Lictravy, Ukraine) at a dose of 16 ml/kg of infusion per day [12], which was prepared according to the rules for the preparation of infusions, which is given in the instructions for medical use in the subclause method of administration [13].

Study object and its dose and preparation before administration

Tested Atherophyton, compressed in 1 tablet of 850 mg contains such medicinal plant raw material: Buckwheat flowers (*Fagopyrum sagittatum* L.) - 85 mg; Periwinkle leaves - 85 mg (*Vinca minor* L.); Lingonberry leaves (*Vaccinium vitis-idaea* L.) - 85 mg; Rose hips (*Rosa canina* L.) - 85 mg; Birch leaves (*Betula pendula* L.) - 85 mg; Buckthorn bark (*Rhamnus frangula* L.) - 50 mg; Pine needles (*Pinus* L.) - 50 mg; Hawthorn flowers (*Crataegus sanguinea* Pall.) - 43 mg; Leuzea carthamoides rhizomes with roots (Rhapónticum carthamoídes) - 43 mg; Sweet

clover herb (*Melilotus officinalis* L.) - 43 mg; Horse chestnut seeds (*Aesculus hippocastanum* L.) - 43 mg; Peppermint leaves (*Mentha piperita* L.) - 43 mg; Horsetail herb (*Equisetum arvense* L.) - 43 mg; Columns with corn tips (*Zea mays* L.) - 43 mg [9].

The dose of the tested PPC was selected based on the recommendation for the use of the dietary supplement indicated on the product packaging [9] (the average therapeutic dose for a person weighing 70 kg is 4250 mg per day: 4250 mg / 70 kg = 60.70 mg/kg, respectively, taking into account the species sensitivity coefficient, the dose for animals is: 60.70 mg/kg - 0.45, X mg/kg - 1.89. X = 255 mg/kg of dietary supplement per day) [12].

Drugs comparing their dose and preparation before administration

The comparison drug metformin was administered at a dose of 60 mg/kg of animal weight, which was also calculated using the species sensitivity coefficient: the average daily dose for a 70 kg human is 1000 mg/day; 1000 mg/70 kg = 14.3 mg/kg for humans. The daily dose for rats and Syrian golden hamsters is 14.3 mg/kg - 0.45, X mg/kg - 1.89. X = 60 mg/kg of metformin per day [12]. The comparison preparation, Arfazetin, contains the following medicinal plant raw materials: Common bilberry shoots (*Vaccinium myrtillus* L.) - 0.2 g, Common bean fruit flaps (*Phaseolus* L.) - 0.2 g, Eleutherococcus prickly rhizomes and roots (*Eleutherocyccus senticysus*) - 0.15 g, Rose hips (*R. majalis* Herrm.) - 0.15 g, Horsetail herb (*Equisetum arvense* L.) - 0.1 g, St. John's wort herb (*Hypericum perforatum* L.) - 0.1 g, Chamomile flowers (*Chamomilla recutita* L.) - 0.1 g per 1.0 g of packaged collection. The dose of Arfazetin was selected based on the recommendations for the dosage of tinctures and the instructions for medical use of the drug (the average therapeutic dose for a person weighing 70 kg is 260 ml of infusion per day: 260 ml/70 kg = 3.7 ml/kg, respectively, taking into account the species sensitivity coefficient, the dose for animals is: 3.7 ml/kg - 0.45, X ml/kg - 1.89. X = 16 ml/kg of infusion per day) [12]. The infusion was prepared daily ex temporis and administered warm [13].

Determination of the content of low-grade inflammatory markers

The following indicators were markers of low-grade inflammation: IL-1 β, IL-6, and TNF-α. The concentration of these cytokines was determined using an immune-enzyme assay with the set of reagents Vector-Best Firm (Russian Federation).

Statistical analysis

Describe the statistical software used to collect data and perform analysis, statistical methods used, sample size calculation and statistical threshold accepted. Statistical processing was performed using Statistica 12.5 (StatSoft, Inc., USA), and the normality of the distribution was checked using the W-Shapiro-Wills test. The data were found to be non-normally distributed, so the non-parametric Mann-Whitney U test was used, and the results were presented as median (Me) and interquartile range (25–75 percentiles). The accepted significance level was $p<0.05$. To obtain statistical conclusions, we used the standard program "package Statistica" [14].

Results and discussion

Against the background of EMS development, low-grade inflammation was observed, as evidenced by an increase in the level of pro-inflammatory cytokines in the blood serum: IL-1 β by 2.5 times ($p<0.05$); IL-6 by 3.4 times ($p<0.05$) and TNF-α by 3.3 times ($p<0.05$) (Table 1). Our findings correlate with the results of other researchers [15–18] who also found the presence of low-grade inflammation in the setting of EMS.

During low-grade systemic inflammation, adipose tissue, muscle tissue, and the liver are infiltrated by inflammatory macrophages that produce TNF-α. This cytokine directly interferes with the ability of these tissues to respond to insulin [19]. IL-6 is one of the cytokines released by both macrophages and adipocytes [20] and has been shown in scientific sources to increase insulin resistance and obesity. IL-6 regulates fat and glucose metabolism by mediating insulin resistance through various complex mechanisms [21]. This cytokine acts on various tissues; in the liver, IL-6 increases the production of acute phase reagents, including C-reactive protein. IL-6 also contributes to prothrombotic conditions by increasing the fibrinogen level, another acute phase reactant [6]. In addition, IL-6 targets other tissues, such as vascular smooth muscle cells and endothelial cells, to promote the expression of vascular cell adhesion molecules and activation of local biochemical pathways, leading to vascular wall atherosclerosis, inflammation and dysfunction [21, 22]. Pro-inflammatory cytokine IL-1β mediates the expression of a large number of genes involved in secondary inflammation, can induce arginine vasopressin secretion and contributes to the risk of cardiovascular

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Table 1: The effect of Atherophytone and comparison drugs on the level of pro-inflammatory cytokines in the blood serum against the background of EMS modeling.

Terms of the experiment/ parameter under study	IL-1 β, PCG/ml	IL-6, PCG/ml	TNF-α, PCG/ml
NC	24.11 (23.39; 24.41)	8.45 (8.14; 9.03)	12.93 (11.75; 13.21)
EMS	60.10 * (59.22; 60.87)	29.00 * (27.74; 29.94)	42.78 * (42.32; 45.03)
EMS + Atherophyton, 255 mg/kg	39.32 * @ # ψ (39.04; 40.00)	16.40 * @ # ψ (15.72; 16.90)	25.76 * @ # ψ (25.07; 26.84)
EMS + Metformin, 60 mg/kg	28.88 * @ € ψ (28.72; 30.07)	11.50 * @ € ψ (10.87; 11.97)	21.53 * @ € ψ (19.22; 22.37)
EMS + Arfazetin, 16 ml/kg	44.98 * @ # € (44.25; 46.23)	21.76 * @ # € (21.01; 23.15)	34.23 * @ # € (32.87; 34.87)

Note: NC – normal (intact) control; EMS – experimental metabolic syndrome; * – reliably in relation to normal (intact) animals (IC), $p<0.05$; @ – reliably in relation to EMS, $p<0.05$; # – reliably in relation to metformin-treated animals, $p<0.05$; ψ – reliably in relation to Arfazetin-treated animals, $p<0.05$; € – reliably in relation to Atherophyton-treated animals, $p<0.05$.

disease and type 2 diabetes in patients with metabolic syndrome [23]. Other sources indicate that IL-1β is a key cytokine induced by metabolic stress [24] along with IL-6 and can cause and exacerbate insulin resistance and metabolic disorders [25]. Recently, the production and secretion of IL-1β have also been reported from pancreatic islets. The insulin-producing β-cells in the pancreatic islets are particularly susceptible to destruction and loss of function under the influence of IL-1β. Macrophage production of IL-1β in insulin-sensitive organs leads to inflammation progression and insulin resistance induction in obesity [15].

The use of Atherophytone contributed to a decrease in cytokine levels of IL-1β, IL-6 and TNF-α by 1.5 ($p<0.05$), 1.8 ($p<0.05$) and 1.7 ($p<0.05$) times, respectively. Under the influence of the comparison drug metformin, the content of interleukins decreased by 2.1 (IL-1β, $p<0.05$), 2.5 (IL-6, $p<0.05$) and 2.0 (TNF-α, $p<0.05$) times, respectively. The use of Arfazetin contributed to a decrease in cytokine levels of IL-1β, IL-6 and TNF-α by 1.3 ($p<0.05$), 1.3 ($p<0.05$) and 1.25 ($p<0.05$) times, respectively (Table 1). Regarding the effect on all the low-grade inflammatory markers studied, the investigational drug Atherophytone was superior to the comparison drug Arfazetin and inferior to Metformin.

Recently, there has been increasing evidence that atherogenesis may be a consequence of a meta-inflammatory process of vascular damage, in which plaque formation leads to atherosclerosis and ultimately to serious events such as myocardial infarction, cerebral vascular disease or sudden death [26–28]. It is systemic inflammation, along with dyslipidemia, that is identified as a crucial factor in atherogenesis and vascular risks [15]. Therefore, the ability of a promising drug to significantly suppress the subchronic inflammatory process, which was established experimentally for Atherophyton in this study, and to correct dyslipidemia, as stated by the manufacturer of the dietary supplement [9], is a winning application point in the pharmacocorrection of the cluster symptom complex that is the metabolic syndrome. The manifestation of the pharmacological effect of DS Atherophyton is due to the complex composition of the active components of the investigated herbal remedy, which includes biologically active substances from 14 medicinal plants and which were obtained by cryotechnology [8], which allows to preserve and increase the concentration of biologically active substances of the original plant material by reducing technological operations and using low temperatures during grinding. Cryo-grinding technology allows the preservation of the maximum amount of biologically active substances and virtually avoids their destruction. As a result of the application of cryo-technology, we obtain fine and extremely fine powders with a large specific surface area, which affects the rate of biochemical reactions in the human body [8] and, accordingly, the pharmacological effect of the investigated product Atherophyton.

Conclusion

The research results show that DS Atherophyton can suppress the development of low-grade inflammation

in experimental metabolic syndrome in Syrian golden hamsters.

Conflict of interest

The authors declare no conflict of interest.

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