https://doi.org/10.31925/farmacia.2023.2.5 **ORIGINAL ARTICLE**

FRUCTOSE INDUCED CHANGES IN RAT SERUM LIPIDS AND ADIPOSE TISSUE GENE EXPRESSION AND PREVENTIVE EFFECTS OF *AGRIMONIA EUPATORIA* AQUEOUS INFUSION INTAKE

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Abstract

Potential of various herbs in regard to their ability to counteract obesity and related metabolic disturbances has been extensively studied. The aim of the current study was to assess the potential of *Agrimonia eupatoria* aqueous infusion (AE) to counteract fructose induced changes in serum total cholesterol (TC) and triglycerides (TG), the ratio of retroperitoneal adipose tissue weight/body weight and adipose tissue gene expression of the proteins *Lipe*, *Fasn*, *Dgat*, *Hmgcr* and adipokines *AdipoQ* and *Plin*. Twenty-four male Wistar rats were equally divided in to four groups - standard diet (ST); standard diet with AE (ST+AE); fructose overloaded (FR); fructose overloaded with AE (FR+AE). FR group showed significantly higher levels of TC, TG and adipose tissue weight relative to other groups. AE administration resulted in lower TC and TG levels, as compared to ST group (0.93 ± 0.43 mmol/L *vs*. 1.29 ± 0.57 mmol/L, $p < 0.05$ and 0.59 ± 0.1 *vs*. 0.91 ± 0.17 mmol/L, $p < 0.001$, respectively). Similarly, TC and TG were decreased also in ST+AE group (0.47 ± 0.21 mmol/L *vs*. 0.87 ± 0.57 mmol/L, $p < 0.01$ and 0.77 ± 0.15 *vs*. 0.92 ± 0.25 mmol/L, $p < 0.05$, respectively) in comparison to ST group. Gene expression analysis revealed a significant increase in *Lipe* (2.35 fold change, $p < 0.05$) and *Hmgcr* (2.86 fold change, $p < 0.05$) and a decrease (83%, $p < 0.001$) in *Fasn* mRNA levels in the FR group. AE consumption contributed to a significant decrease in the expression of *Dgat2* (56%, $p < 0.05$) and *Hmgcr* (0.52%, $p < 0.05$) in Fr+AE group and of *Fasn* (71%, $p < 0.001$) in the ST+AE group. In conclusion, recent study demonstrated that *Agrimonia eupatoria* aqueous infusion intake improved total cholesterol and triglycerides imbalance in *Wistar* rats and modulated adipose tissue gene expression, which indicated that the herb might exert its favourable effects on adipose tissue possibly by preventing triglyceride synthesis and inducing mobilization of stored TG.

Rezumat

În general, potențialul terapeutic al diferitelor plante pentru combaterea obezității și al dezechilibrelor metabolice asociate este intens studiat. Scopul prezentului studiu a fost de a evalua capacitatea infuziei apoase de *Agrimonia eupatoria* (AE) de a echilibra modificările induse de fructoză asupra valorilor colesterolului total (TC) și trigliceridelor (TG) din ser, de a echilibra raportul masă țesut adipos retroperitoneal/masă corporală, și de a modula expresia genelor pentru proteinele *Lipe*, *Fasn*, *Dgat*, *Hmgcr* și adipokinele *AdipoQ* și *Plin* din țesutul adipos. Douăzeci și patru de șobolani albi Wistar au fost împărțiți în patru loturi, care au primit următoarele diete: dietă standard (ST); dietă standard cu AE (ST+AE); supliment de fructoză (FR); supliment de fructoză cu AE (FR+AE). Lotul FR a prezentat nivele semnificativ crescute ale TC, TG și ale masei de țesut adipos, comparativ cu celelalte loturi. Administrarea de AE a condus la o scădere a valorilor TC și TG comparativ cu lotul ST ($0,93 \pm 0,43$ mmol/L față de $1,29 \pm 0,57$ mmol/L, $p < 0,05$ și, respectiv $0,59 \pm 0,1$ față de $0,91 \pm 0,17$ mmol/L, $p < 0,001$). În mod similar, au scăzut valorile TC și TG și în cazul lotului ST+AE *versus* ST ($0,47 \pm 0,21$ mmol/L *versus* $0,87 \pm 0,57$ mmol/L, $p < 0,01$ și, respectiv $0,77 \pm 0,15$ *versus* $0,92 \pm 0,25$ mmol/L, $p < 0,05$). Analiza expresiei genelor a evidențiat o creștere semnificativă a *Lipe* (de 2,35 ori, $p < 0,05$) și *Hmgcr* (de 2,86 ori, $p < 0,05$) și o scădere a concentrațiilor ARNm corespunzător *Fasn* (83%, $p < 0,001$) pentru lotul FR. Consumul de AE a contribuit în mod semnificativ la scăderea expresiei genelor *Dgat2* (56%, $p < 0,05$) și *Hmgcr* (0,52%, $p < 0,05$) în cazul lotului FR+AE și a genei *Fasn* (71%, $p < 0,001$) pentru lotul ST+AE. În concluzie, studiul a demonstrat că administrarea infuziei apoase de *Agrimonia eupatoria* la șobolanii Wistar normalizează valorile colesterolului total și ale trigliceridelor serice și modulează expresia genelor la nivelul țesutului adipos, fapt ce arată că planta poate exercita efecte favorabile asupra metabolismului lipidic, probabil prin blocarea sintezei de trigliceride și prin inducerea mobilizării TG stocate.

Keywords: fructose, *Agrimonia eupatoria*, agrimony, lipids, gene expression

Introduction

It is known that fructose overload due to its wide use in food and drinks provoke insulin resistance, impaired lipid metabolism, disturbed adipose tissue metabolism and adipocytokines secretion and even obesity and type 2 diabetes [2, 13, 30, 34]. Fructose abuse models

are used in animal studies to investigate fructose induced metabolic changes and underlying molecular mechanisms. For example, fructose supplementation has been reported to increase blood TC and TG content, as well as adipose tissue hypertrophy and body weight gain [1, 3, 4, 8, 35]. Tissue gene expression profiles also significantly changed representing its effect on overall metabolism and physiology [14, 15, 25]. Adipose tissue is important target of fructose effects and gene expression changes indicate adipogenic consequences and impaired signalization and lipid metabolism [37, 38].

The potential of various herbs in regard to their ability to counteract obesity and related metabolic disturbances has been extensively studied in recent years. Identification of herbal remedies and/or phyto-chemicals with anti-obesity properties would contribute to development of functional foods for prevention and treatment of this condition. In our previous studies we have established anti-obesity potential of agrimony (*Agrimonia eupatoria* L.) in humans and in rats [26] and impact on gene expression in cell cultures [19, 21]. *A. eupatoria* has also been established to be rich in polyphenols and other biologically active substances with antioxidant and other biological properties [18].

The aim of the current study was to assess the potential of *A. eupatoria* aqueous infusion to counteract fructose induced changes in the serum lipids – total cholesterol (TC) and triglycerides (TG), changes in adipose tissue weight and adipose tissue gene expression of proteins related to lipid metabolism - hormone-sensitive lipase (*Lipe*), fatty acid synthase (*Fasn*), diacylglycerol acyl-transferase 2 (*Dgat2*), 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (*Hmgcr*), and adipokines- adiponectin (*AdipoQ*) and perilipin (*Plin*).

Materials and Methods

Animal models

Male albino Wistar rats were housed at a 20 ± 2ºC room temperature and with a standard 12 h light/dark cycle with *ad libitum* access to water. Animals were fed with standard diet composed of 50% starch, 20% protein, 4.5% fat, 5% cellulose, standard vitamins and mineral mix. At age of two months and 140 - 180 g body weight, animals were divided into four groups (n = 6 each): standard diet fed rats (ST); standard diet fed rats administered with agrimony water extract (AE) (ST+AE); fructose overloaded rats (FR); fructose overloaded rats administered with AE (FR+AE). Fructose overload was achieved by adding 12.5% fructose in drinking water. Animals were grown for additional 12 weeks when samples were collected under lethal dose of thiopental. The weight of retro-peritoneal fat was measured. Adipose tissue was washed with PBS (pH = 7.4) and stored at -80ºC until gene expression analysis. Serum samples were collected and analysed the same day.

The experimental procedures were approved by the Home Office for Care and Use of Laboratory Animals and performed with a strong consideration for ethics of animal experimentation according to the International Guiding Principles for Animal Research approved in Bulgaria [40]..

Biochemical analysis of total cholesterol (TC) and triglycerides (TG)

Serum TC and TG content was measured with commercially available kits (BioMaxima, S. A. Poland).

Plant material and extract preparation

Commercial product of Selibum Ltd, Varna, Bulgaria, standardized according to the European Pharmacopoeia was tested in the study. Aqueous infusion was prepared according the following ratio recipe - 2.5 g of the plant material infused with 200 mL boiling water for 10 minutes.

Gene expression analysis

RNA extraction was performed as follows: approximately 30 mm^3 (20 mg) frozen adipose tissue were smashed immediately in 500 µL TRIzol reagent (Ambion, Austin, TX, USA) in 1.5 sterile tubes. Further the procedure followed as described by manufacturer. Isolated RNA was reversely transcribed with RevertAid First Strand cDNA Synthesis Kit (ThermoScientific, Waltham, MA, USA) using (dT)18 primer. Reaction was performed according to the manufacturer's guidelines in a final volume of 10 µL. Gene expression analysis was performed using qReal-Time PCR method. Beta actin was used as endogenous control. Primer sets were as follows:

Actb F: GGGAAATCGTGCGTGACATT;
Actb R: GCGGCAGTGGCCATCTC;
Dgat2 F: ACAGTGGGTCCTATCCTTCC,
Dgat2 R: ATCTCCTGCCACCTTTCTTG;
Fasn F: GGCCTGGAGTCTATCATCAA;
Fasn R: CTGCACTCAGGGTGTGAT;
AdipoQ F: CCGTGATGGCAGAGATGG;
AdipoQ R: CACCCTTAGGACCAAGAACAC;
Plin F: TCCTGTTCGGCATCTCTTTAC;
Plin R: TTTGTACTGACATAAGCGGAGG;
Lipe F: CTACAGGACTATGTCACGCTAC;
Lipe R: GGATGGCAGGTGTGAACT;
Hmgcr F: AACCTGCTGCCATAAACTGGAT;
Hmgcr R: ACCACCTTGGCTGGAATGAC.

Gene expression levels were calculated according the $2^{-\Delta\Delta Ct}$ method [24] and were presented in relative units as compared to a control group, where expression is considered to be equal to 1.

Statistical analysis

The values of biochemical parameters were represented as average of a minimum of three measurements ± SD and as a mean ± SEM for gene expression analysis. Comparison between groups was performed using *t*-test and differences with p value < 0.05 were considered as significant. Data processing was performed using

the statistical software product Graph Pad Prism (Ver. 5.0 Graph Pad Software, Inc.).

Results and Discussion

Serum lipid parameters – total cholesterol (TC) and triglycerides (TG)

Possibilities to prevent and/or treat metabolic disturbances with a proper diet have been of interest considering natural medicine application and functional foods development. In this regard the potential of medicinal plants has been studied and remains not fully elucidated. In our previous studies we have established anti-obesity potential of agrimony in humans and rats [26].

Agrimonia eupatoria L. is a wide distributed herb, prescribed in folk medicine for the treatment of inflammatory diseases and as an antidiabetic remedy [29]. It is rich in variety of biologically active substances. A recent review [28] provides a summary about its tannins, flavonoids, phenolic acids, triterpenoids, fatty acids, volatile oils, vitamin and mineral content. Phytochemical characterization reveals that components such as epigallocatechin, hydroxyphenylacetate, ferulic acid, caffeic acid and especially neohesperidin and epicatechin predominate in aerial part extract [17]. Leaves are also rich in agrimoniin, apigenin-7-*O*-glucuronide and apigenin-6-*C*-glucoside [22].

The aim of this study was to assess the possibility to modulate with agrimony tea intake serum lipid parameters and adipose gene expression in a model of rats supplemented with fructose. In addition to high-lipid, extensively used to trigger metabolic impairments, including chronic inflammation [11], fructose supplementation has also been extensively used in animal models to induce metabolic disturbances and dyslipidaemia. Fructose supplementation is widely used to assess possible protective potential of various natural products, including antioxidants and medicinal plants [7, 16]. In this study we applied 12-week supplementation with 12.5% fructose. The concentration was used in previous studies and has been approved to induce significant changes in body weight and metabolic disturbances [6].

Although fructose supplementation has been reported to lead to increase blood TC and TG content [1, 4, 8, 35] in our study 12-week overload with 12.5% fructose in drinking water did not contribute to significant changes in these parameters (Figure 1a and Figure 1b).

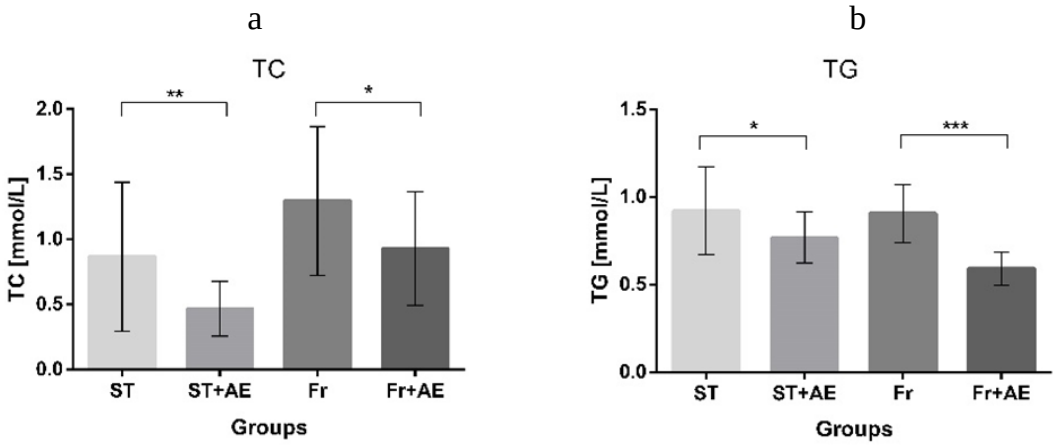

Figure 1.

Total cholesterol (TC) and triglycerides (TG) content in serum of male Wistar rats administered with AE tea for period of 12 weeks in a model of fructose supplementation

ST – standard diet; ST+AE – standard diet and AE; Fr – 12.5% Fructose supplementation; Fr+AE – 12.5% Fructose supplementation and AE

AE intake appeared to have significant lowering effect on serum TC (0.47 ± 0.21 mmol/L *vs*. 0.87 ± 0.57 mmol/L, $p < 0.01$ and 0.93 ± 0,43 mmol/L *vs*. 1.29 ± 0.57 mmol/L, $p < 0.05$) and TG (0.77 ± 0.15 *vs*. 0.92 ± 0.25 mmol/L, $p < 0.05$ and 0.59 ± 0.1 *vs*. 0.91 ± 0.17 mmol/L, $p < 0.001$) both in standard diet and fructose overloaded rats with AE intake, respectively (Figure 1a and Figure 1b). Improvement of lipid profile has been attributed to medicinal plants. For example, Shahraki *et al*. [32] established prevention of TC and TG increase in fructose fed rats supplemented with aqueous extract of *Tamarindus indica* seeds. Similarly, *Commiphora mukul* ethanolic extract prevents plasma TG elevation in fructose fed experimental animals [3]. Findings of present study in agreement with other reports indicate the potential of medicinal plants as a remedy for improvement of lipid profile parameters in case of impaired TC and TG blood levels [5]. In humans, *A. eupatoria* tea intake resulted in improved lipid plasma profile [20]. Neohesperidin rich extracts were reported to decrease TG and TC content in 3T3-L1 adipocytes, *Caenorhabditis elegans* worms and plasma in mice [33]. Similarly, epicatechin significantly reduced TC and TG and alleviated liver fat accumulation in hyperlipidaemic rats [10].

Gene expression of Lipe, Fasn, Dgat, Hmgcr, AdipoQ and Plin

Possible preventive effect of AE was examined in rat adipose tissue in a fructose abuse model by evaluating the gene expression levels of selected proteins – lipid metabolism related (*Lipe* – a lipolytic enzyme responsible for triacylglycerols hydrolysis in adipose tissue; *Fasn* – involved in the synthesis of fatty acids; *Dgat2* – in TG synthesis; *Hmgcr* – the rate limiting enzyme in *de novo* cholesterol synthesis) and adiponectin (*AdipoQ*) and perilipin (*Plin*). Established mRNA levels, calculated on the basis of ST as a control, in Fr and Fr+AE groups are presented in Figure 2.

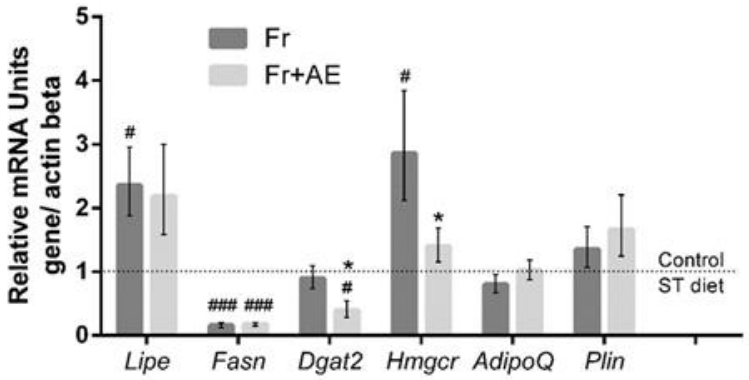

Figure 2.

Expression levels of *Lipe*, *Fasn*, *Dgat2*, *Hmgcr*, *AdipoQ* and *Plin* in adipose tissue of male Wistar rats administered with AE tea for period of 12 weeks in a model of fructose supplementation. Gene expression levels are presented in relative units ± SEM, calculated on the basis of ST group as a control, where gene expression levels of each gene of interest equals 1 according to the $2^{-\Delta\Delta Ct}$ method. * *vs*. Fr group; # *vs*. ST group

Table I

Fold change in mRNA levels of *Lipe*, *Fasn*, *Dgat2*, *Hmgcr*, *AdipoQ* and *Plin* in AE administered fructose fed rats as compared to the fructose fed rats

Gene	*Lipe*	*Fasn*	*Dgat2*	*Hmgcr*	*AdipoQ*	*Plin*
Change (%)	↓0.7% ns	↑12% ns	↓56% $p < 0.05$	↓52% $p < 0.05$	↑27% ns	↑22% ns

In order to evaluate the effect of AE in fructose fed rats in fold change we calculated mRNA levels using Fr group as a control, where gene expression levels of each gene of interest equalled 1 according to the $2^{-\Delta\Delta Ct}$ method. Data are presented in Table I.

Gene expression analysis revealed significantly up-regulated *Lipe* and *Hmgcr* gene expression (2.35 and 2.86 fold change, respectively, $p < 0.05$) and approximately 83% down-regulated one of *Fasn* – 0.17 relative units in Fr group, compared to ST group ($p < 0.001$). As the fructose rich diet is commonly considered to be associated with increased cholesterol levels, increased expression of *Hmgcr* seems meaningful, although adipose tissue might not be the main tissue contributing to total cholesterol levels in blood. Additionally, elevated level of serum TC was also detected in the Fr group (Figure 1a). As liver is considered to play a central role in cholesterol metabolism, most reports refer to this tissue where a stimulation of *Hmgcr* has been reported [7, 27, 38]. Data about adipose tissue *Hmgcr* expression changes in fructose overload state are scarce and indicate rather lower mRNA levels, as compared to standard diet fed animals [38]. In this study we established decreased *Hmgcr* expression in adipose tissue of AE administered fructose supplemented rats. Increased levels of *Lipe* were detected in rat adipose tissue in our study. This finding is in accordance with Bursać *et al*. [9], who report that fructose consumption enhances glucocorticoid action in rat visceral adipose tissue and thus may stimulate glucocorticoid targeted genes. Similarly, in cell culture, 3T3-L1 adipocytes differentiated in fructose-containing medium had elevated lipolysis and enhanced expression of hormone sensitive lipase and adipocyte triglyceride lipase, and elevated release of glycerol and free fatty acids [23], suggesting also a potent adipocyte differentiation property of fructose *via* increasing local glucocorticoid activation. Insulin-induced inhibition of liver lipolysis did not occur in mice administered with fructose [31]. *Lipe* activation might also be associated with adipose tissue remodelling, which also leads to its hypertrophy and increased mass. In our experiment, we found a significant increase in adipose tissue relative to body weight in the fructose-fed group (Figure 3a and Figure 3b). We presume that the adipose tissue reduction by means of AE is possibly due to changes in genes associated with the activity of lipid metabolism and energy balance.

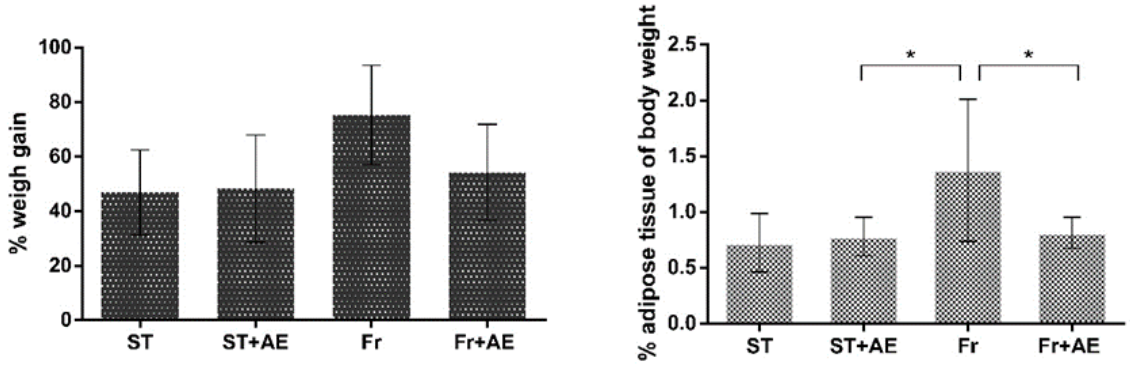

Figure 3.
Body weight gain (%) and adipose tissue (% of body weight) in Wistar rats after in a model of 12 weeks of fructose supplementation. * < 0.05

Fructose has been found to favour increased fatty acid synthesis in adipocytes in a dose dependent manner [36]. In contrast, in our experiment *Fasn* expression was decreased in rat adipose tissue, while in a similar model, microarray analysis did not reveal significant change in adipose *Fasn* while a significant increase in liver and a decrease in skeletal muscle, were found representing a tissue specific response [38]. Similarly, in Wistar rats adipose tissue *Fasn* was downregulated significantly by fructose overload [12].
AE supplementation resulted in about 56% lower *Dgat2* mRNA levels in fructose fed rats ($p < 0.05$) in comparison to the Fr group (Table I). In this group (Fr+AE) *Dgat2* expression was significantly lower even than in ST diet fed rats, estimated in 61% lower mRNA units ($p < 0.05$) (Figure 2). Although down-regulated, *Dgat2* was not significantly decreased in ST diet fed rats, supplemented with AE, indicating that this herbal remedy might have stronger effect on *Dgat2* in conditions of fructose overload. *Fasn* mRNA levels in Fr+AE group were lower than the ST group (Figure 2), but the effect was probably due to fructose, as no significant difference between Fr and Fr+AE was established. However, AE supplementation significantly decreased this gene expression in ST diet fed rats by 71% ($p < 0.001$) (Figure 4). Similarly, grape seed flour causes decrease in Fasn in white adipose tissue, both in normal and fructose diet fed mice [39].

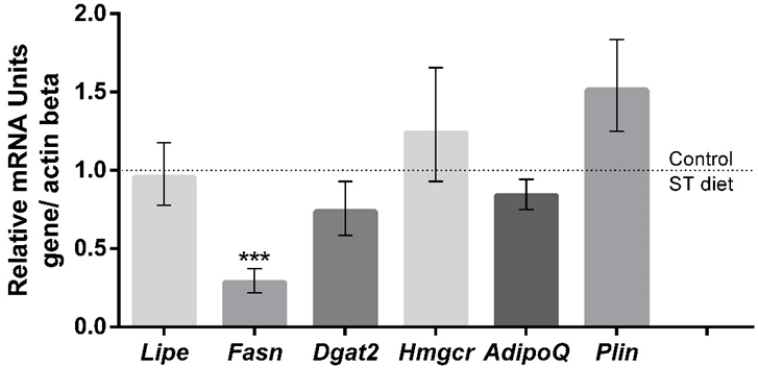

Figure 4.
Expression levels of *Lipe*, *Fasn*, *Dgat2*, *Hmgcr*, *AdipoQ* and *Plin* in adipose tissue in AE administered male Wistar rats for a period of 12 weeks
Gene expression levels are presented in relative units ± SEM, calculated on the basis of ST group as a control, where gene expression levels of each gene of interest equals 1 according to the $2^{-\Delta\Delta Ct}$ method. * *vs*. ST group

AdipoQ and *Plin* did not show any significant changes in this experiment in neither group. Probably the effect of fructose and AE did not involve an interaction with regulatory systems of their expression on transcriptional level.
Agrimony extracts are especially rich in neohesperidin and epicatechin [17] that are contained in plant formulations found to significantly reduce expression of genes related to lipid metabolism and adipocyte differentiation in adipocyte cell culture and experimental animal models [10, 33].

Conclusions

Our study demonstrated that *Agrimonia eupatoria* aqueous infusion intake improved total cholesterol and triglycerides imbalance induced by fructose

supplementation in Wistar rats. Gene expression data also suggest that the herb exerts its favourable effect on adipose tissue possibly by downregulating triglyceride and cholesterol synthesis and inducing mobilization of stored TG in conditions of fructose overload - in support to the concept of adipose tissue as a possible therapeutic target for nutraceuticals.

Conflict of interest

The authors declare no conflict of interest.

References

1. Aguilera-Mendez A, Hernández-Equihua MG, Rueda-Rocha AC, Guajardo-López C, Nieto-Aguilar R, Serrato-Ochoa D, Ruíz Herrera LF, Guzmán-Nateras JA, Protective effect of supplementation with biotin against high-fructose-induced metabolic syndrome in rats. *Nutr Res.*, 2018; 57: 86-96.
2. Basciano H, Federico L, Adeli K, Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr Metab (Lond.)*, 2005; 2(1): 5.
3. Bellamkonda R, Karuna R, Sasi Bhusana Rao B, Haritha K, Manjunatha B, Silpa S, Saralakumari D, Beneficiary effect of *Commiphora mukul* ethanolic extract against high fructose diet induced abnormalities in carbohydrate and lipid metabolism in Wistar rats. *J Tradit Complement Med.*, 2017; 8(1): 203-211.
4. Benado M, Alcantara C, de la Rosa R, Ambrose M, Mosier K, Kern M, Effects of various levels of dietary fructose on blood lipids of rats. *Nutr. Res.*, 2004; 24(7): 565-571.
5. Bratoeva K, Bekyarova G, Kiselova Y, Ivanova D, Effect of Bulgarian herb extracts of polyphenols on metabolic disorders-induced by high fructose diet. *Trakia J Sci.*, 2010; 8(2): 56-60.
6. Bratoeva K, Bekyarova G, Kiselova Y, Radanova M, Ivanova D, Metabolic changes in experimental model of metabolic syndrome - induced by high-fructose diet in rats. *Scr Sci Med.*, 2010; 42(4): 233-235.
7. Bratoeva K, Nikolova S, Merdzhanova A, Stoyanov GS, Dimitrova E, Kashlov J, Conev N, Radanova M, Association between serum CK-18 levels and the degree of liver damage in fructose-induced metabolic syndrome. *Metab Syndr Relat Disord.*, 2018; 16(7): 350-357.
8. Bratoeva KZ, Radanova MA, Merdzhanova AV, Donev IS, Protective role of s-adenosylmethionine against fructose-induced oxidative damage in obesity. *J Mind Med Sci.*, 2017; 4: 163-171.
9. Bursać BN, Djordjevic AD, Vasiljević AD, Milutinović DD, Veličković NA, Nestorović NM, Matić GM, Fructose consumption enhances glucocorticoid action in rat visceral adipose tissue. *J Nutr Biochem.*, 2013; 24(6): 1166-1172.
10. Cheng H, Xu N, Zhao W, Su J, Liang M, Xie Z, Wu X, Li Q, (-)-Epicatechin regulates blood lipids and attenuates hepatic steatosis in rats fed high-fat diet. *Mol Nutr Food Res.*, 2017; 61(11): 1700303-14.
11. Dimitrov I, Kamenov V, Boyadjiev N, Georgieva K, Bivolarska A, Draganova-Filipova M, Angelova P, Delchev S, Daskalova E, Gerginska F, Stankova T, Gramatikov V, Impact of a high-fat diet on the development of chronic inflammation in heart of Wistar rats. *Folia Medica*, 2019; 61(3): 404-410.
12. Dziadek K, Kopeć A, Piątkowska E, Leszczyńska T, High-fructose diet-induced metabolic disorders were counteracted by the intake of fruit and leaves of sweet cherry in Wistar rats. *Nutrients*, 2019; 11(11): 2638.
13. Elliott SS, Keim NL, Stern JS, Teff K, Havel PJ, Fructose, weight gain, and the insulin resistance syndrome. *Am J Clin Nutr.*, 2002; 76(5): 911-922.
14. Gonçalves NG, Cavaletti SH, Pasqualucci CA, Arruda Martins M, Lin CJ, Fructose ingestion impairs expression of genes involved in skeletal muscle's adaptive response to aerobic exercise. *Genes Nutr.*, 2017; 12: 33.
15. Gonzalez JT, Symposium overview: fructose in physiology. *J Physiol.*, 2019; 597(14): 3523-3525.
16. Hernandez E, Portillo S, Anaya O, Valle M, Flores J, Chavez R, Galindo G, Mondragon L, Cobos, D, Guerrero G, Sanchez P. Renoprotective and hepatoprotective effects of *Hippocratea excelsa* on metabolic syndrome in fructose-fed rats. *Farmacia*, 2020; 68(6): 1106-1119.
17. Huzio N, Grytsyk A, Raal A, Grytsyk L, Koshovyi O, Phytochemical and pharmacological research in *Agrimonia eupatoria* L. herb extract with anti-inflammatory and hepatoprotective properties. *Plants*, 2022; 11(18): 2371-2388.
18. Ivanova DG, Tasinov OB, Vankova DG, Kiselova-Kaneva YD, *Agrimonia eupatoria* L. extract modulates glutamate-cysteine ligase and glutathione peroxidase expression in 3T3-L1 cells. *Bulg J Agric Sci.*, 2013; 19(2): 171-174.
19. Ivanova DG, Tasinov OB, Vankova DG, Kiselova-Kaneva YD, Antioxidative potential of *Agrimonia eupatoria* L. *Sci Technol Med.*, 2011; 1(1): 20.
20. Ivanova D, Vankova D, Nashar M, *Agrimonia eupatoria* tea consumption in relation to markers of inflammation, oxidative status and lipid metabolism in healthy subjects. *Arch. Physiol Biochem.*, 2013; 119(1): 32-37.
21. Karlińska E, Romanowska B, Kosmala M, The Aerial Parts of *Agrimonia procera* Wallr. and *Agrimonia eupatoria* L. as a Source of Polyphenols, and Especially Agrimoniin and Flavonoids. *Molecules*, 2021; 26(24): 7706-7727.
22. Kiselova YD, Vankova DG, Tasinov OB, Feklicheva I, Potoroko I, Ivanova DG, Changes in interleukin 6 and monocyte chemoattractant protein-1 expression levels in 3t3-l1 cells after exposure to *Agrimonia eupatoria* L. extract and subsequent oxidative stimulation with tert-buthyl hydroperoxide reveal possible adaptogenic potential. *Scr Sci Pharm.*, 2017; 2: 7-13.
23. Legeza B, Marcolongo P, Gamberucci A, Varga V, Bánhegyi G, Benedetti A, Odermatt A, Fructose, Glucocorticoids and Adipose Tissue: Implications for the Metabolic Syndrome. *Nutrients*, 2017; 9(5): 426.
24. Livak KJ, Schmittgen TD, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*, 2001; 25(4): 402-408.
25. López-Rodríguez G, Osuna SK., Galván MG, Suárez-Diéguez T, Effects of dietary high fructose corn syrup on regulation of energy intake and leptin gene

expression in rats. *Rev Nutr Campinas*, 2015; 28: 597-605.
26. Nashar M, Kiselova-Kaneva Y, Ivanova D, in: Nutrition in Health and Disease-Our Challenges Now and Forthcoming Time, Gyula Mózsik and Mária Figler, IntechOpen, 2019; 1.
27. Oishi K, Konishi T, Hashimoto C, Yamamoto S, Takahashi Y, Shiina Y, Dietary fish oil differentially ameliorates high-fructose diet-induced hepatic steatosis and hyperlipidemia in mice depending on time of feeding. *J Nutr Biochem.*, 2018; 52: 45-53.
28. Paluch Z, Biriczová L, Pallag G, Carvalheiro Marques E, Vargová N, Kmoníčková E, The therapeutic effects of *Agrimonia eupatoria* L. *Physiol Res.*, 2020; 69 (Suppl 4): S555-S571.
29. Pamukov D, Ahtardziev H, Prirodna apteka/Natural Pharmacy. Zemizdat: Sofia, Bulgaria, 1989; 427.
30. Rutledge AC, Adeli K, Fructose and the Metabolic Syndrome: Pathophysiology and Molecular Mechanisms. *Nutr Rev.*, 2007; 65: 13.
31. Sato T, Watanabe Y, Nishimura Y, Inoue M, Morita A, Miura S, Acute fructose intake suppresses fasting-induced hepatic gluconeogenesis through the AKT-FoxO1 pathway. *Biochem Biophys Rep.*, 2019; 18: 100638.
32. Shahraki MR, Harati M, Shahraki AR, Prevention of high fructose-induced metabolic syndrome in male Wistar rats by aqueous extract of *Tamarindus indica* seed. *Acta Med Iran*, 2011; 49(5): 277-283.
33. Shen S, Wan L, Wang T, Jiang J, *Citrus aurantium* L. var. *amara* Engl. inhibited lipid accumulation in 3T3-L1 cells and *Caenorhabditis elegans* and prevented obesity in high-fat diet-fed mice. *Pharmacol Res.*, 2019; 147: 104347-104359.
34. Stanhope KL, Havel PJ, Fructose consumption: potential mechanisms for its effects to increase visceral adiposity and induce dyslipidemia and insulin resistance. *Curr Opin Lipidol.*, 2008; 19(1): 16-24.
35. Thirunavukkarasu V, Anitha Nandhini AT, Anuradha CV, Effect of alpha-lipoic acid on lipid profile in rats fed a high-fructose diet. *Exp Diabesity Res.*, 2004; 5(3): 195-200.
36. Varma V, Boros LG, Nolen GT, Chang CW, Wabitsch M, Beger RD, Kaput J, Metabolic fate of fructose in human adipocytes: a targeted 13C tracer fate association study. *Metabolomics*, 2015; 11(3): 529-544.
37. Zhang YH, An T, Zhang RC, Zhou Q, Huang Y, Zhang J, Very high fructose intake increases serum LDL-cholesterol and total cholesterol: a meta-analysis of controlled feeding trials. *J Nutr.*, 2013; 143(9): 1391-1398.
38. Zhang H, Shen WJ, Li Y, Bittner A, Bittner S, Tabassum J, Cortez YF, Kraemer FB, Azhar S, Microarray analysis of gene expression in liver, adipose tissue and skeletal muscle in response to chronic dietary administration of NDGA to high-fructose fed dyslipidemic rats. *Nutr Metab (Lond.)*, 2016; 13: 63.
39. Zhou F, Yin M, Liu Y, Han X, Guo J, Ren C, Wang W, Huang W, Zhan J, You Y, Grape seed flour intake decreases adiposity gain in high-fat-diet induced obese mice by activating thermogenesis. *J Funct Food*, 2019; 62: 103509.
40. https://bfsa.egov.bg/wps/portal/ bfsa-web/activities/animal.health.and.welfare/humanno-otnoshenie/test-animals