

Oral administration of *Arctium tomentosum* Mill. CO₂-extract alleviates the diet induced metabolic disorder in mice

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Abstract. Nowadays, the metabolic disorder exists along with other pathological conditions, such as hyperglycemia, hypertriglyceridemia, hypercholesterolemia and gain of the excess abdominal fat. It has negative impact on human health, but especially the liver health. The present work was designed to study the liver supporting effect of ATE on mice with high-fructose and high-sucrose diet induced metabolic disorder. The study was conducted on mice, divided into following groups: NC; untreated MD; group with MD treated by ATE at the doses 50, 200 and 400 mg/kg respectively. As a result, the liver supporting effect of ATE at the doses of 200 and 400 mg/kg was shown in the values of body weight, relative weight of the liver, kidneys and epididymal fat. The same was observed in plasma biochemistry, in which groups treated with ATE showed normal levels of triglycerides, total and low-density cholesterol, ALT, AST. The treatment by 400 mg/kg ATE significantly inhibited the lipid accumulation and hepatocytes degeneration in the liver histological structure. In conclusion these findings provide the potential therapeutic usefulness into the effects of ATE in the treatment of metabolic disorder. Furthermore, it suggests that ATE has hepatoprotective effect which is conducted via its antioxidant properties.

1 Introduction

Nowadays, the metabolic disorder (MD) exists along with other pathological conditions, such as hyperglycemia, hypertriglyceridemia, hypercholesterolemia and gain of the excess abdominal fat. It has negative impact on human health, but especially the health of the liver [1]. Most of the hepatic diseases are derived, or are quite connected to the presence of the metabolic damage [2]. Long-term damage of the glucose metabolism leads to the development of glucose intolerance and insulin-resistance, which then increase the probability of the type 2 diabetes development [3]. Abnormal lipid metabolism, elevated levels of triglycerides and cholesterol along with high-blood pressure increase the probability of cardiovascular disease development [4]. Since the liver is responsible for the abundance of important functions, including blood sugar balance, cholesterol conversion and fatty acids

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storage, altogether these conditions put hard workload on the liver [4]. Long-term overburdened liver cannot appropriately regulate metabolism, causing hormonal disbalance, abdominal fat storage and central obesity [5]. Consequently, the liver support can alleviate these symptoms, also preventing development of more severe hepatic conditions, such as fat liver and liver cirrhosis [6]. Some conventional chemical drugs can protect liver from damage, however most of them are derived from synthetic molecules, that are not supposed to circulate in the human body [7,8]. Moreover, the chemical drugs are metabolized by the liver and kidneys, what creates an additional load to these organs [9,10]. Thus, using of medicinal plants is an alternative and cost-available way of medical treatment. *Arctium tomentosum* Mill. is a ruderal species that has a high content of biologically active compounds with antioxidant, antimicrobial and anti-inflammatory properties [11-15]. However, no studies on the liver supporting and protecting effect of *A. tomentosum* extract (ATE) were conducted yet. The present work designed to study the liver supporting effect of ATE on mice with high-fructose and high-sucrose diet induced metabolic disorder.

2 Materials and Methods

2.1 Plant material

Woolly burdock roots were collected from Aksai gorge (Almaty, Kazakhstan) and identified to be the roots of *Arctium tomentosum* Mill. by Prof. Meruert Kurmanbayeva of the al-Farabi Kazakh National University. *A. tomentosum* CO₂-extract was prepared according to the Republic Standard Procedure No. 8050-85.

2.2 Animal Procedures

The studies were conducted on mice, six weeks old, of 19.2 ± 0.7 g body weight. The animals originated from and were maintained throughout the experiment in the Animal Facility of Scientific Center for Anti-Infectious Drugs at the standard laboratory conditions. Twenty-five mice were used in the experiment. Initially, animals were initially divided into following groups: first – negative control (NC), (n=5) and second – MD model (n=20).

The MD model was induced by 30% high-fructose and 30% high-sucrose diet (Sigma-Aldrich, Massachusetts, USA) for eight weeks. After eight weeks this group was divided into four groups: MD model and three ATE groups. The MD model group remain untreated, while three ATE groups were orally treated by doses of 50, 200, 400 mg/kg until Week 15. The experiment conduction was approved by the Ethical Committee of Scientific Center for Anti-Infectious Drugs, No. 23/15 in accordance with the ARRIVE guidelines.

2.3 Sample Collection and Storage

Animals were fasted for 12h, then euthanized by isoflurane anesthesia. The blood was collected from retroocular sinus on clot activator containers until biochemical study, then the liver and kidneys were removed and weighed. Some parts of liver specimens were placed and stored in pre-cooled 10% formaldehyde until histological study. The commercial assay kits were used to detect the levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), alaninaminotransferase (ALT) and aspartataminotransferase (AST).

2.4 Histology

The samples of liver tissue were fixed in 10% formaldehyde after collection. Then the pieces of liver were dehydrated in ethyl alcohol and embedded in paraffin wax. The histological blocks were then sliced and stained by H&E (Sigma-Aldrich, Massachusetts, USA). Then the histological examination was conducted under microscope Carl Zeiss Axio Scope.A1 (ZEISS, Germany). The liver tissue inflammation and fat distribution were analyzed to make microscopic photographs.

2.5 Statistical analysis

The results were analysed using the Graph Pad Prism 6.0 statistical software. The values are expressed as the mean $\pm$ SD. Control and test groups comparison was evaluated through one-way ANOVA following by Bonferroni post-test done. Statistical significance was set at $p < 0.05$.

3 Results

The values of body weight, relative weight of the organs (liver, kidney, epididymal fat) are presented in the Figure 1.

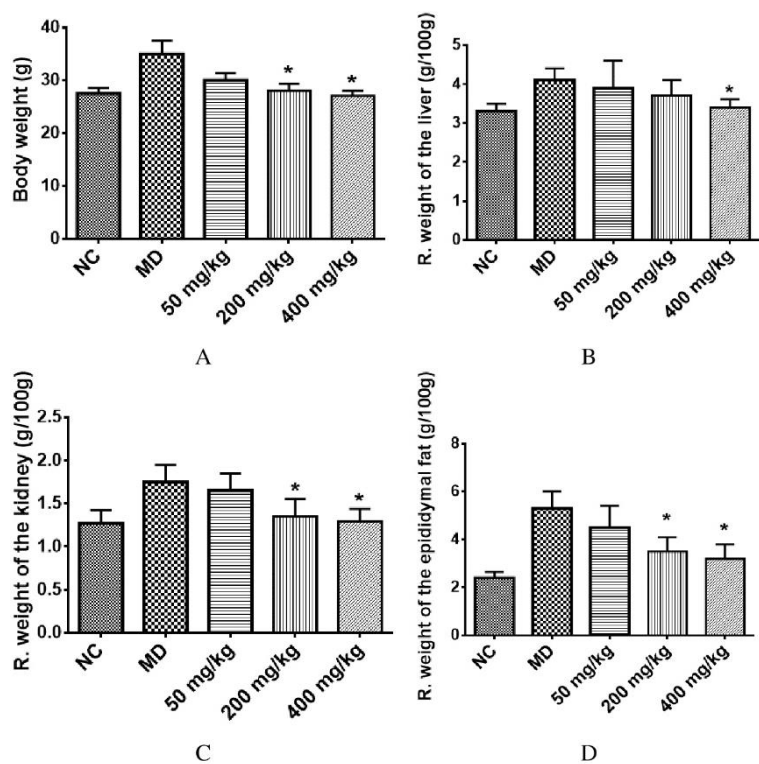


Fig. 1. The values of body weight (A); relative weight of the liver (B), kidneys (C) and epididymal fat (D)

Mice with diet induced MD had strong tendency of body fat gain until the end of the experiment. It can explain the role of metabolic damage in the development of central obesity. The ATE group of mice, oppositely showed inhibition of weight gaining process (Figure 1A).

The relative weight of the liver and kidneys were also increased in MD group, in comparison with group treated by ATE at the doses of 200mg/kg and 400 mg/kg (Figure 1B-D), while administration of ATE suppressed the epididymal fat gain ($p<0.05$)*. ATE effect on levels of glucose and plasma biochemistry is presented in the Figure 2.

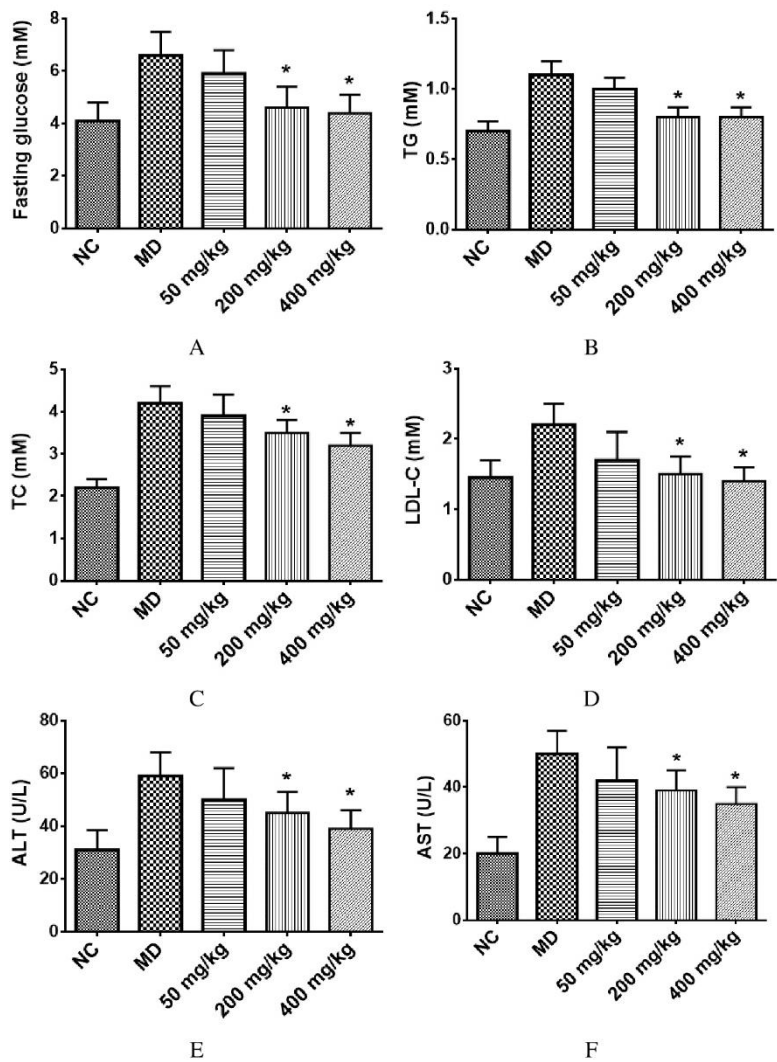


Fig. 2. The levels of fasting glucose (A), TG (B), TC (C), LDL-C (D), ALT (E) and AST (F), * $p<0.05$

Mice with diet induced MD had increased levels of fasting glucose in blood serum (Figure 2A). Plasma biochemistry in mice with MD was generally manifested as high levels of triglycerides, total cholesterol, low-density lipoprotein cholesterol, alaninaminotransferase and aspartataminotransferase (Figure 2B-F), indicating liver insufficiency and abnormal lipid metabolism. The ATE treated group showed some alleviation of these symptoms, indicating that the treatment was effective ($p<0.05$).

In order to study the liver supporting effect of ATE on MD derived liver injury, the histological examination was conducted. Microphotographs made during histological examination are presented in the Figure 3.

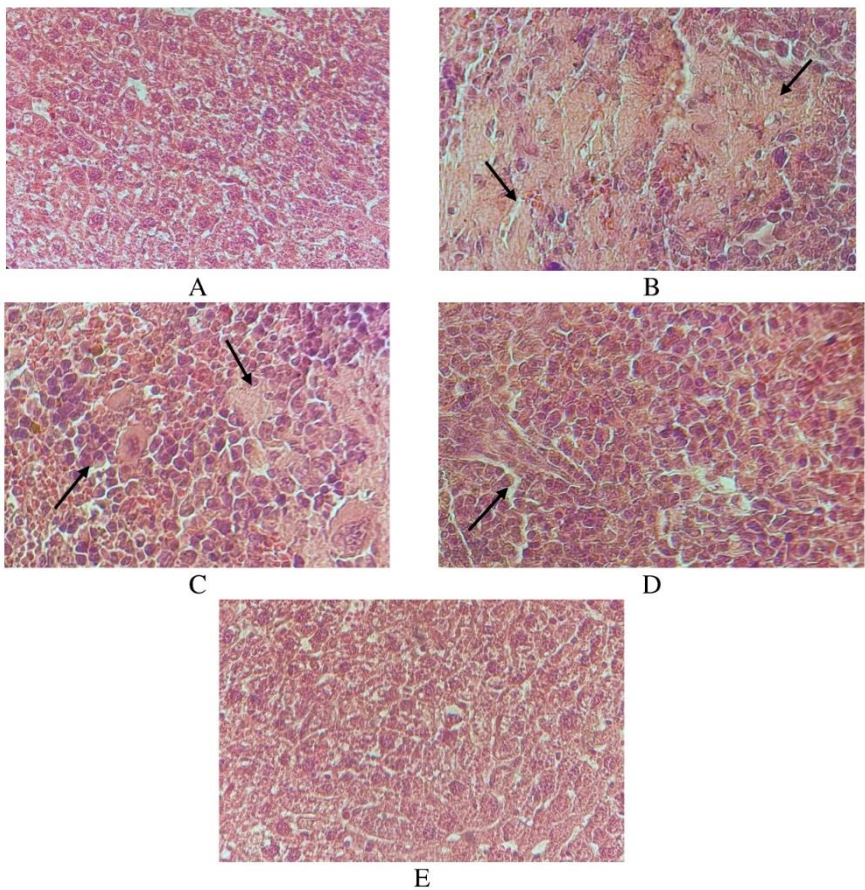


Fig. 3. Effect of ATE on liver histological structure: NC (A), MD (B), ATE 50 mg/kg (C), ATE 200 mg/kg (D), ATE 400 mg/kg (E). H&E; 100 μ m

In the result of histological examination vigorous lipid accumulation was observed in the MD model group (Figure 3B). Mild amelioration was observed in mice treated by 50 mg/kg ATE; however, degeneration of hepatocytes was still presented (Figure 3C). The treatment at the doses of 200 mg/kg and 400 mg/kg ATE showed more evident alleviation of histopathological processes (Figure 3D, E).

Thus, the liver supporting effect was more evident at higher doses of 200 mg/kg and 400 mg/kg, showing strong effect to the values of body weight, relative weight of the liver, kidneys and epididymal fat. The same was observed in plasma biochemistry, in which groups treated with ATE showed normal levels of TG, TC, LDL-C, ALT and AST. Also, treatment with ATE at the dose of 400 mg/kg significantly inhibited the lipid accumulation and hepatocytes degeneration in the liver histological structure.

4 Conclusion

The effects of ATE treatment was studied in mice with the diet induced metabolic disorder at the doses of 50, 200 and 400 mg/kg. The parameters of body weight, weight of the liver, kidneys and epididymal fat were significantly different between MD model groups and treated groups. The same tendency was observed in the fasting glucose level and plasma

biochemistry parameters. Also ATE treatment showed liver supporting effect during histological examination, alleviating hepatic necrosis and fat liver development. In conclusion these findings provide the potential therapeutic usefulness into the effects of ATE in the treatment of metabolic disorder. Furthermore, it suggests that ATE has hepatoprotective effect which is conducted via its antioxidant properties.

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