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THE PHYTOTHERAPY EFFECT ON LIVER'S CYTOARCHITECTURE OF HIGH-FAT DIET RATS TREATED WITH STATINS

EFECTUL FITOTERAPIEI ASUPRA CITOARHITECTURII FICATULUI ȘOBOLANILOR CU DIETA BOGATĂ ÎN GRĂSIMI TRATAȚI CU STATINE

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ABSTRACT | REZUMAT

Remedies that lower hyperlipidaemia helps to avoid cardiovascular problems. In this regard, statins are agents that have hypolipidemic properties; Atorvastatin is the drug of choice used for the management of hyperlipidaemia and cardiac diseases. But, like other remedies, it has some adverse effects, mainly on the liver that occur within months of medication commencement. In this study, we tried to evaluate the effect of a High-Fat Diet (HFD) on the liver histology of rats receiving atorvastatin. Also the phototherapeutic activities of Sea buckthorn (*Hippophae rhamnoides L.*) (SBT) and grape (*Vitis vinifera L.*) extracts as hepatoprotective agents was observed. One hundred and twelve white Wistar rats were included, divided into seven groups and given the following oral doses: GI, atorvastatin 20 mg; GII, atorvastatin 20 mg + SBT 100 mg; GIII, atorvastatin 20 mg + grape extract 100 mg; GIV, grape extract 100 mg; GV, SBT extract 100 mg; HFD and normal diet (C) groups. After two and six months, respectively, the rats were euthanized and the liver organs were gathered for cytoarchitecture examination. The liver's cytoarchitecture in GI revealed an abnormal cytoarchitecture. In GII, GIII, GIV, and GV, cell regeneration improved in different stages, especially for GII and GIII compared with the HFD group, which exhibited fat degeneration. The findings confirm the hypothesis that dietary supplements, such as SBT and grape, are excellent hypolipidemic agents. Therefore, they can be considered a beneficial resource for reducing hyperlipidemia and improving the fatty liver cytoarchitecture produced by HDF when given with or without drugs in hyperlipidaemic cases.

Keywords: statins, hepatotoxicity, cytoarchitecture, phytotherapy, Sea-buckthorn, grapes

Remediile care scad hiperlipidemia ajută la evitarea problemelor cardiovasculare. In acest sens, statinele sunt agenți care au proprietăți hipolipemiante; atorvastatina este medicamentul de elecție utilizat pentru gestionarea hiperlipidemiei și a bolilor cardiace. Dar, ca și alte remedii, are unele efecte adverse, in principal asupra ficatului, care apar in câteva luni de la începerea tratamentului. In acest studiu, am încercat să evaluăm efectul unei diete bogate in grăsimi (HFD) asupra histologiei ficatului la șobolanii cărora li s-a administrat atorvastatină. In plus, au fost observate activitățile fitoterapeutice ale extractelor de cătină și de struguri ca agenți hepatoprotectori. In acest studiu au fost incluși 112 șobolani albi Wistar, împărțiți în șapte grupuri la care s-au administrat următoarele doze orale: GI atorvastatină 20 mg, GII atorvastatina 20 mg + SBT 100 mg, GIII atorvastatina 20 mg + extract de struguri 100 mg, GVI extract de struguri 100 mg, GV extract SBT 100 mg, HFD și grupul Control (C). După două, respectiv șase luni, șobolanii au fost eutanasiați și ficatul a fost prelevat pentru examinarea citoarhitecturii. Structura ficatului a relevat o citoarhitectură anormală. În GII, GIII, GIV și GV regenerarea celulară s-a îmbunătățit în diferite etape, în special pentru GII și GIII, comparativ cu grupul HFD, care a prezentat o degenerare lipidică. Studiul nostru confirmă ipoteza că suplimentele alimentare folosite sunt agenți hipolipemianți excelenți. Prin urmare, pot fi considerate o resursă benefică pentru reducerea hiperlipemiei și îmbunătățirea citoarhitecturii hepatice grase produsă de o dietă bogată în grăsimi, când sunt administrate cu sau fără medicamente.

Cuvinte cheie: statine, hepatotoxicitate, citoarhitectura, fitoterapie, cătină, struguri

Hyperlipidaemia is a medical condition characterized by abnormal elevation of any or all lipid profiles, with or without lipoproteins in the bloodstream. The consumption of lipids that raise body fat over time has increased dramatically as a change in lifestyle. A high-fat diet (HFD) may cause complications related to lipid metabolisms, such as hyperlipidaemia, insulin resistance, and visceral obesity (2).

It has been proven that high levels of plasma Total cholesterol (TC) and Low-density lipoprotein-c (LDL-*c*)

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cause atherosclerosis in humans, and many researchers suggest that high levels of plasma High-density lipoprotein-*c* (HDL-*c*) protect against atherosclerosis. Hypercholesterolemia promotes atherosclerosis and is a major cardiovascular disease (CVD) risk factor (13, 18). So, reducing cholesterol and LDL-c levels describes the golden point for the prevention of primary and secondary stages of CVD (13, 17, 18).

The lipid-lowering remedy is indicated for primary and secondary management of hyperlipidaemia and CVD, in addition to the control of all other risk factors, including obesity, diabetes, and smoking (17). Statins and fibrates are modern antihyperlipidemic therapy. The former corrects the abnormal blood lipid profile by decreasing cholesterol biosynthesis, while the latter improves triglyceride-rich lipoprotein clearance (3). Statins 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase inhibitors are a group of cholesterol-lowering agents applied to manage hyperlipidaemia and reduce the risk of atherosclerotic cardiovascular disease (14, 15). They are the main prescribed remedies worldwide, and the drug of choice due to their potent influences on the lipid profile with cholesterol-independent cardio-protective outcomes (1,14). In comparison to untreated hypercholesterolemic patients, statins have been demonstrated to reduce or even reverse the progression of coronary atherosclerosis, resulting in fewer new injuries and obstructions (3, 21). Despite their widespread usage, discontinuation and non-adherence remain a significant gap in both the primary and secondary restrictions of atherosclerotic CVDs. The critical statin side effects include the onset of type II diabetes mellitus, perhaps a haemorrhagic stroke, neurological and neurocognitive consequences, hepatotoxicity, renal toxicity, urogenital, reproductive, and gastrointestinal problems (3, 8, 10, 15, 17, 26).

Atorvastatin belongs to the statin group, which has been linked to hepatotoxicity in recent decades, with various hepatotoxicities that happens after several months of receiving the first dose of the drug (10). The specific mechanism by which it causes liver injury is not well known. Phytotherapy, which includes Phenolic compounds (PCs) and natural antioxidants, is regarded as a reliable option for avoiding medicine's adverse effects. Plants are considered important reservoirs for the antioxidant substances, already identified to be essential in the protection of the cardiovascular system against injuries (5, 11, 12, 19, 21, 23-25).

Moreover, medicinal plants have played a significant part in the treatment of a variety of illnesses. They contain natural substances that can improve health and alleviate disease. Natural products and herbal formulations have supported the development of some of the most important therapeutic medications utilized in modern medicine for health-promoting purposes. So, there is an important requirement to recognize those at heightened risk of developing side effects besides providing alternative therapy strategies (4, 5, 9, 11, 16, 19, 20, 22-25).

Sea buckthorn (*Hippophae rhamnoides L.*) belongs to the family *Elaeagnaceae*. It has been used for medical and nutritional purposes. The berries contain several bioactive compounds like carotenoids, vitamins, antioxidants, flavonoids, essential fatty acids, amino acids, and phytosterols (4). In comparison to an SBT-free diet, SBT supplementation has been shown to lower total cholesterol, triglyceride, and LDL-*c* levels while increasing HDL-*c* levels (12, 23-25).

The grape (*Vitis vinifera*), a species that belongs to the *Vitaceeae* family, is a non-climatic fruit that grows all over the world. It is rich in polyphenol chemicals, which are beneficial to human health and chronic disease models (20, 22). Its extract is a mixture of polyphenolics such as oligomeric proanthocyanidins and unsaturated fatty acids that is commonly used as a dietary supplement.

Polyphenols' health benefits may be linked to their antioxidant, vasodilator, and antihypertensive activities, as well as their involvement in lowering serum cholesterol levels and preventing atherogenic plaque. It has shown that the consumption of polyphenol at high levels lowers the risk of CVD (9).

MATERIALS AND METHODS

Ethical approval

The research was approved by the Ethical Committee of the Faculty of Veterinary Medicine from Banat's University of Agriculture Science and Veterinary Medicine from Timisoara, Romania, no. 6/30.01.2019. Before starting, rats were accommodated for ten days to accommodate the laboratory conditions and were handled under Directive 2010/63/EU on the handling of animals used for scientific purposes (7).

Animals and the experimental design

To evaluate the protective effects of phytotherapy concerning hyperlipidaemia induced by HFD, 112 white Wister rats were included, males and females, weighing between 150–165 g, and aging 3–4 months. Animals were procured from the National Research Institute for Microbiology and Immunology "Cantacuzino" (Bucharest, Romania). The experimental animals were housed in polycarbonate cages and supplied *ad libitum* with a standard diet for rodents.

The animals were kept at 22 ±2 °C, and 55 ±10% humidity. During the research period, the light cycle was of 12 light/12 dark hours. The rats were divided into seven experimental groups and orally dosed as follows: **GI** received 20 mg × kg^{-1} B.W. atorvastatin; **GII** received a combination of 20 mg × kg^{-1} B.W. ator-

vastatin + 100 mg × kg^{-1} B.W. SBT; **GIII** received a combination of 20 mg × kg^{-1} B.W. atorvastatin + 100 mg × kg^{-1} B.W. grape extract; while **GIV** and **GV** received 100 mg × kg^{-1} B.W. SBT and grape extract respectively.

The **HFD** group was considered to control positive while the **normal** group was considered a control negative. All groups were fed with HFD (19, 20), except the control group, which was fed a standard diet for rodents for six months of therapy. Rats were sacrificed, eight/group/period, and liver organs were gathered to investigate the liver's cytoarchitecture after two and six months of the remedial doses.

Atorvastatin

Atorvastatin belongs to the class of statins that are used to lower cholesterol levels, maintain plaque, and prevent strokes by acting on anti-inflammatory and other mechanisms. It works by lowering LDL-*c* by inhibiting HMG-CoA reductase (21). It was purchased from Help Net pharmacy, Timisoara, Romania, and used as an oral suspension in distilled water at a dose of 20 mg × kg^{-1} B.W.

Cytoarchitecture examination

The tissue samples were fixated for seven days in 80 percent alcohol, then rinsed in distilled water and dehydrated by immersing in increasing concentrations of ethanol. Xylene was used instead of ethanol, and the samples were hardened in paraffin (Merck, Germany). 5mm slices were cut using a cut 4062 microtome (Mainz, Germany), put on slides, and stained using the Trichromic (TC) stain procedure. Microscopy was performed at ×50 and ×200 magnifications on a CX 41 microscope (Olympus, Germany), including a digital camera and QuickPhoto-Micro2.2 software (Promicra, Czech Rep.) for the images' interpretation.

RESULTS AND DISCUSSIONS

Total polyphenols content (TPC) and individual polyphenols

Table 1 presents the results regarding the individual polyphenols identified in the SBT and Antioxivita extracts. Of the 11 determined polyphenols were identified 5 compounds (rutin, rosmarinic acid, resveratrol, quercetin, kaempferol). Other compounds (gallic acid protocatechuic acid, caffeic acid, epicatechin, p-coumaric acid, ferulic acid) were not detectable (concentration under the limit of detection) (16).

SBT contains rosmarinic acid (43.742 µg/L), quercetin (40.534 µg/L) and resveratrol (28.345 µg/L) and in lower concentration kaempferol (6.208 µg/L). Antioxivita contains higher concentration of quercetin (203.798 µg/L) and kaempferol (270.556 µg/L), but lower of rosmarinic acid (26.271 µg/L) and resveratrol (18.615 µg/L) (16).

Cytoarchitecture results

The histological examination of the liver from the control group revealed the normal architecture of this organ. Normal hepatocytes are disposed of in hepatic cords with a radial arrangement around central vein. Microscopic examination of the liver after the fatty diet (HFD group) pointed out the presence of steatosis to different degrees, expressed by the presence in the hepatocyte cytoplasm of the lipid droplets, which give them a vesicular aspect. After two months of therapy, the number of hepatocytes with small vesicular aspect 'ballooning degeneration' starts to increase in comparison with a normal diet. Also, the installation of steatosis is more emphasized in males than in females. After six months, the fatty degeneration is more severe compared with the last two months (Fig. 1).

Table 1

Individual polyphenols and LC parameters of the hydroalcoholic extracts

Compounds	Retention time (min)	Concentration SBT extract (µg/L)	Concentration Antioxivita extract (µg/L)	Calibration curve
Gallic acid	4.826	nd*	nd	y=8.470·e-006x (r=0.9996)
Protocatecuic acid	11.774	nd	nd	y= 8.036·e-006x (r=0.9990)
Caffeic acid	21.480	nd	nd	y=7.110·e-006x (r=0.9990)
Epicatechin	22.606	nd	nd	y=3.881·e-005x (r=0.9996)
p-coumaric acid	24.737	nd	nd	y=1.1566·e-006x (r=0.9997)
Ferulic acid	24.183	nd	nd	y=1.172·e-006x (r=0.9999)
Rutin	24.183	nd	7.525	y=1.813 ·e-005x (r=0.9999)
Rosmarinic acid	28.203	43.742	26.271	y=1.018 ·e-006x (r=0.9982)
Resveratrol	30.274	28.385	18.615	y=6.388·e-006x (r=0.9945)
Quercitin	31.521	40.534	203.798	y=1.001·e-005x (r=0.9992)
Kaempferol	34.810	6.208	270.556	y=3.273·e-005x (r=0.9990)

nd* (not detectable)

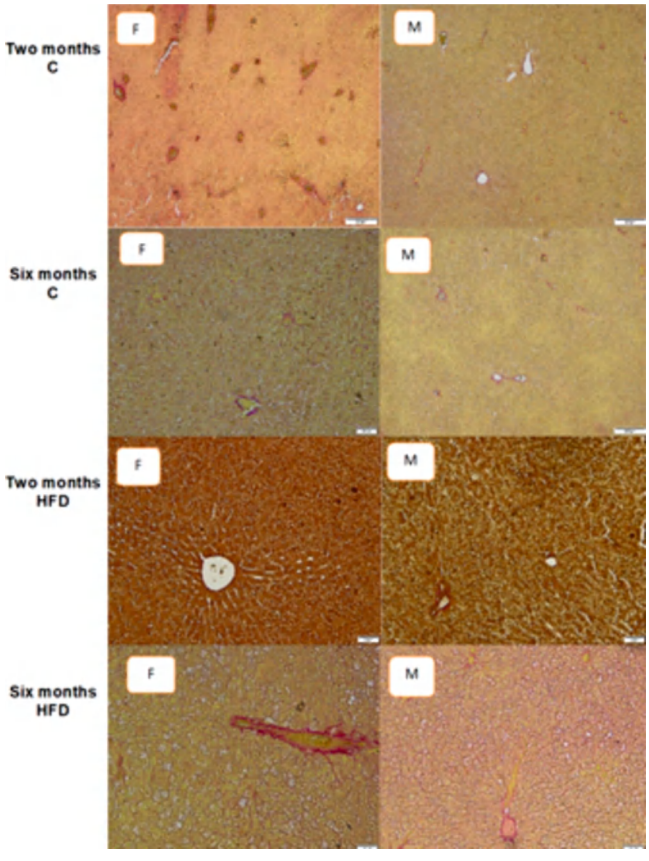

Fig. 1. Histological section through female (F) and male (M) rats' liver (C and HFD) groups after two and six months of treatment. TC stain. Scale bar: 50, 200 μm

After two months of treatment with 20 mg/kg^{-1} B.W., the microscopical examination of the female rat liver revealed the presence of abnormal hepatocytes with vacuolar cytoplasm, with pyknotic nuclei, and karyolysis, localized between normal hepatocytes with uniform, compact cytoplasm and round, central nuclei. The absence of lipid droplets was noticed. While, in males the signs of steatosis exist, with large vesicular hepatocytes localized at the periphery of the hepatic lobules, compared with the control group, where the alternative injuries were just starting to express (Fig. 2). The combination of 20 mg atorvastatin with 100 mg × kg^{-1} B.W. SBT (GII) and 100 mg × kg^{-1} B.W. grape extract (GIII) revealed the normal aspect of liver architecture. The most significant are the signs of cellular recovery from males, especially after six months of therapy in comparison with the HFD group (Fig. 3). Microscopic examination of the liver in GIV treated with 100 mg × kg^{-1} B.W. Antioxivita revealed the presence of small vesicular hepatocytes localized in the periphery of the hepatic lobule in female rats, while in males there were normal sections after two months of treatment. After six months, histological examination revealed low steatosis with ballooning hepatocytes, more evident in males, where the absence of inflammatory infiltration was noticed (Fig. 4).

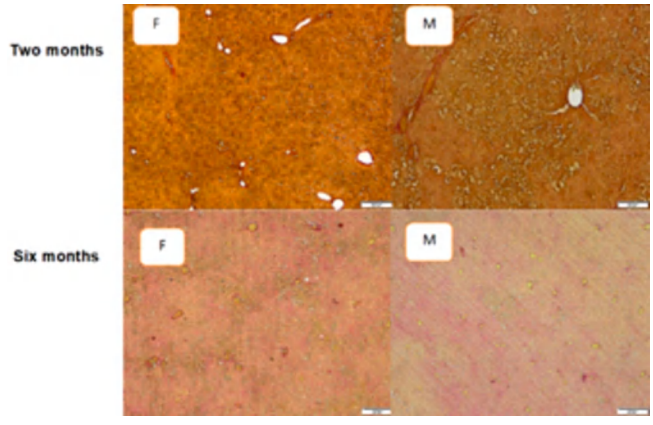

Fig. 2. Histological section through female (F) and male (M) rats' liver (GI) after two and six months of treatment: signs of abnormal hepatocytes small vesicular cytoplasm, pyknotic nuclei, signs of steatosis, cell infiltration, and karyolysis are shown after two months of treatment, while after six months, different size of hepatocytes, inflammatory cell infiltration, ballooning hepatocytes, sinusoidal capillary inflammation are shown. TC stain. Scale bar: 50, 200 μm

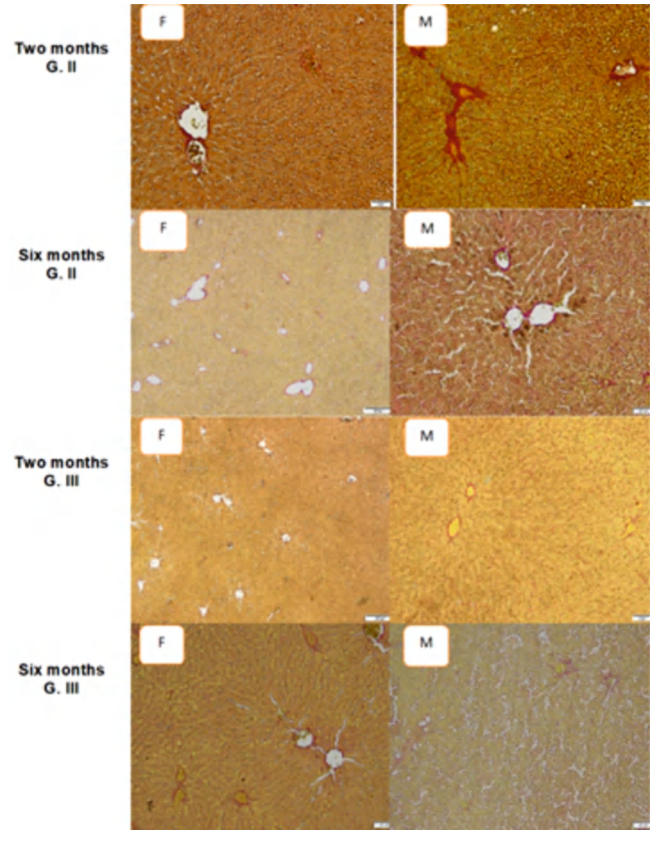

Fig. 3. Histological section through female (F) and male (M) rats' liver (GII and GIII) after two and six months of treatment: signs of a slight sinusoid are observed. TC stain. Scale bar: 50, 200 μm

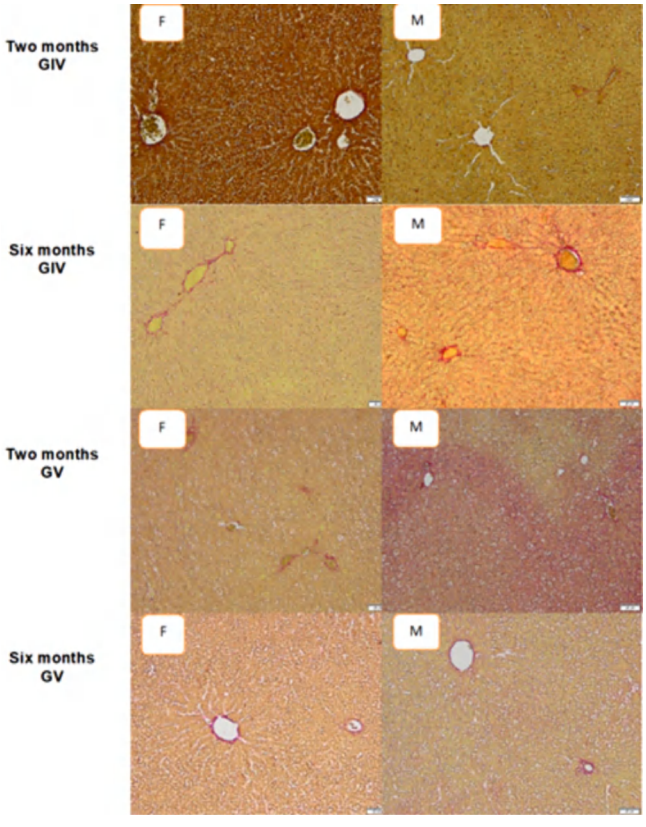

Fig. 4. Histological section through female (F) and male (M) rats' liver (GIV & GV) after two and six months of treatment: normal histological section with low steatosis. TC stain. Scale bar: 50, 200 μm

High-fat diets are widely associated with health complications within the spectrum of metabolic syndrome. Non-alcoholic fatty liver diseases are among the most common of these pathologies, which are generally detected early in the development of HF-dietary problems. Atorvastatin considerably reduced lipid levels due to its inhibition of HMG-Co reductase in microsomal cells, besides its modification of serum LDL-*c* through enhancing LDL-receptor-mediated LDL absorption (6, 14, 18). Abnormal cytoarchitecture, such as hepatocytes with vascular cytoplasm, pyknotic nuclei, and karyolysis confined between normal hepatocytes with uniform, compact cytoplasm, can result from long-term atorvastatin treatment.The signs were much milder in groups treated with a combination of atorvastatin and phytotherapy in GII and GIII. These were followed by healing and the overcome of cellular alterations induced by the HFD over a long period. SBT in GII and GV also has a variety of pharmacological effects, including antioxidant activity due to its high phenol and flavonoid content (20, 24, 25).

While in GIII and GIV, grape extract affected lipoprotein metabolism by lowering lipid production and preventing atorvastatin toxicity due to the presence of high polyphenolic chemicals, there were no vascular hepatocytes or inflammatory infiltrates, which had a hepatoprotective effect against HFD and reduced atorvastatin toxicity. Additionally, several studies have shown that the chemicals found in grapes have beneficial effects on the livers of rats. The ethanol extract of *Vitis vinifera* was found to have a strong protective effect by reducing serum AP and total bilirubin levels (25). Our results are in line with the results of Buchner (5) who found that grape juice consumption can improve oxidative stress and liver damage induced by HFD in rats.

CONCLUSIONS

In conclusion, our findings support the statement that dietary supplements such as SBT and grape extracts taken with or without medicines to treat hyperlipidaemia, were effective in reducing the lipid profile, oxidative damage, and liver enzymes activities caused by their lipid peroxidation features. Plant extracts containing high amounts of polyphenols were found to be effective in protecting the liver and other body organs during the phytotherapeutic therapy period.

Conflict of Interest

There are no conflicts of interest declared by the authors.

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