

Effects of *Berberis vulgaris* Extract on Lipid Profile, Kidney and Liver Function in Experimental Dyslipidemia

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The ability of Berberis vulgaris bark extract (BVE) to improve the lipid profile was evaluated in an experimental model of dyslipidemia, along with its effects on liver and kidney functions. Fifty rats divided into five groups of ten animals each were fed with normal or lard-based diet and orally treated with 0.9% saline solution, atorvastatin (2.5 mg/kg per body weight) or extract of BVE (300 and 500 mg/kg/day) for eight weeks. Lipid profile, liver and renal functions were assessed in normal and diet-induced hypercholesterolemic rats. The results were compared between the groups treated with Berberis extract and the group without treatment, respectively that one treated with the standard drug (atorvastatin). Administration of BVE or atorvastatin significantly decreases the elevated serum lipid profile ($p < 0.05$). The extract also protects against dyslipidemia-induced non-alcoholic fatty liver disease (NAFLD). The activities of the plant extract are dose dependent and it compares favorably with the standard drug atorvastatin. Variations in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and urea level measurements were neither different between the two determinations nor between groups, but creatinine values decreased in rats in the BVE or atorvastatin-treated groups. The findings of the current study have suggested that BVE intake suppresses the accumulation of hepatic lipids and lipid parameters and reduce the risk of NAFLD. So, BVE can be useful in hypercholesterolemia.

Keywords: non-alcoholic fatty liver disease, extract, statins, transaminases, hypercholesterolemia

Cardiovascular disease (CV) represents one of the leading causes of mortality and morbidity worldwide [1]. Dyslipidemia is one of the main risk factors for the development of cardiovascular disorders, such as atherosclerosis and its complications (myocardial infarction, cerebral infarction, peripheral vascular disease) [2]. Approximately 50% of the patients with myocardial infarction and approximately 25% of those with ischemic stroke have high cholesterol levels [3]. The balance between the normal amount of cholesterol and its metabolism is ensured by normal liver function. The liver is the main organ affected by the ingestion of large amounts of lipids [4]. Fat deposition in the liver, in the absence of infection or significant intake of alcohol, characterizes a wide range of liver disorders known as non-alcoholic fatty liver disease (NAFLD) [5]. Moreover, dyslipidemia may contribute to impaired renal function, especially in patients with chronic renal disease (CKD) [6].

An important step in reducing CV risk is the decrease in circulating lipids. Lifestyle changes and pharmacological approaches to lowering cholesterol are widely used in the prevention of CV [7]. HMG-CoA (3-hydroxy-3 methylglutaryl coenzyme A) reductase inhibitors (statins) and fibrates are the most commonly prescribed antidyplipidemic drugs.

These two classes of drugs have better control over dyslipidemia but, although they are very effective agents, they have the disadvantage of hepatic and muscular side effects (increased liver enzymes, rhabdomyolysis) [8, 9].

Nowadays, many researchers are focusing on various medicinal herbs that have an hypolipidemic effect, which can be useful as adjuvants in reducing the risk of cardiovascular disease. Over the past ten years, the promotion of European herbal products has grown. Many have been used in traditional medicine for centuries [10]. *Berberis vulgaris* belongs to the *Berberis* genus, the *Berberidaceae* family. This plant has played an important role in alternative therapy and various parts of this plant (fruits, leaves, roots) have been used in traditional medicine for a long time [11]. Berberine is the most important alkaloid in *Berberis vulgaris*. It is generally stated that this plant has beneficial effects in several disorders (gastrointestinal, cardiovascular, inflammatory and ophthalmic) and many studies have been done so far [12, 13].

Different plants of genus *Berberis*, including *Berberis vulgaris*, grow in many regions of the world including Romania, Europe [14]. In this country, the different parts have been used especially in the treatment of hepatitis and kidney disease [15].

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In this study, we investigated the ability of *Berberis vulgaris* bark extract to improve the altered serum lipid profile in an experimental model of dyslipidemia. We also evaluated the effect of *Berberis vulgaris* on hepatic and renal functions in rats.

Experimental part

Materials and methods

Preparation of Plant Extract

Berberis vulgaris L. was collected from the Alexandru Borza Botanical Garden of Cluj-Napoca, and a voucher specimen (CL 659560) is deposited at the Herbarium of Babes-Bolyai University, Cluj-Napoca, Romania.

The plant extract was prepared by a cold repercolation method at room temperature for 3 days [15, 16] by using small fragments (0.5–1 cm) from stem bark (*Berberidis cortex*) which were extracted with 70% ethanol (Merck, Bucuresti, Romania) in the Mycology Laboratory of Babes-Bolyai University, Cluj-Napoca, Romania. The *B. vulgaris* extract, containing 1 g plant material in 1.6 mL of 35% ethanol (w/v), was obtained by filtration.

Quantification of berberine in *Berberis vulgaris* extract (BVE)

For quantification of berberine in *Berberis* sp. extract, a high-performance liquid chromatography method coupled with mass spectrometry (LC/MS/MS) was used. The LC/MS system was an Agilent 1100 Series HPLC system (Agilent Technology Co., Ltd.) consisting of a binary pump, degasser, autosampler, thermostat operating at 48°C, SL Ion Trap detector and UV detector). The chromatographic separation was performed on a Zorbax SB-C18 column (100mm×3.0mm i.d., 3.5µm) (Agilent) preceded by a 0.5 µm online filter. The mobile phase consisted of acetonitrile and 0.2 % (V/V) formic acid in water, in the ratio 30:70 (V/V) and was delivered at a flow rate of 1 mL/min. The autosampler injection volume was set at 1 µL. The mass spectrometer operated using ESI source in positive mode (dry gas nitrogen, dry gas flow 12 L/min, nebulizer 60 psi) and was set for isolation and fragmentation of berberine molecular ion with $m/z = 336$. Quantification of berberine was based on multiple reaction monitoring mode (the sum of ions with $m/z = 292.1$ and 321.1 from the mass spectrum of berberine). The calibration curve was linear in the range of 51–1654 ng/mL, with a correlation coefficient of 0.9996. The extract was diluted 1:1000 prior analysis. The content of berberine (% w/w) in the ethanolic extracts was expressed as mean±standard deviation (SD). All measurements were performed in triplicates.

Drugs and reagents

The pharmaceutical product was Atorvastatin (40 mg tablets, Actavis, UK). The lard was obtained from pigs raised in Romania and was bought from a single common commercial store. This compound has already been characterized in our country: 100g contains 99.6g% total lipid and 928 calories [18].

Biochemical measurements were performed in a specialized Laboratory of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca.

Animals and protocol

The animals included in this study were Charles River Wistar (n = 50) white male rats weighing between 100 and 200 g, obtained from the Center for Experimental Medicine and Practical Skills (Cluj-Napoca, Romania). The working protocol was revised and approved by the Ethics Committee of the Iuliu Hatieganu University of Medicine

and Pharmacy, Cluj-Napoca, Romania, as well as by the Sanitary Veterinary and Food Safety Direction of Cluj-Napoca.

The rats were kept in cages, in a clean room, with 12 h light/dark cycles and a temperature of $22\pm 2^\circ\text{C}$. For two days before the start of the experiment, the animals were acclimated under these conditions. The Guiding Principles in the Use of Animals in Toxicology adopted by the Society of Toxicology (USA) and the Law 43/2014 regarding the protection of animals used for scientific research published in *Monitorul Oficial* (Romania), were the specific regulations and amendments in this study.

The rats were randomly divided into five groups of ten each: group I served as control (with normal feeding); group II received lard (L) (1.0 mL) and saline solution; group III received L (1.0 mL) and BVE 300 mg/kg; group IV received L (1.0 mL) and BVE 500 mg/kg and group V received L and atorvastatin 2.5 mg/kg. Animals were fed ad libitum with a specific diet of rats, with or without lard for 60 days and received the drug (extract or statin) between day 30 and day 60 of our study. Animals also had free access to tap water.

Blood samples were collected on the 30th day and on the last day, the 60th of the experiment. The periorbital method, from the retroorbital plexus of the rats, was used. After coagulation, the blood was centrifuged and the plasma was separated and stored at -20°C to determine the biochemical markers. Serum samples were tested for triglycerides (TG), total cholesterol (TC), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and urea. Animals were sacrificed by deep anesthesia via ether.

Preparation of liver sections for histological study

At the end of the study, the liver of each animal was removed. Sections of excised liver tissues from all animals were preserved in 10% formaldehyde, dehydrated in graduated ethanol and embedded into paraffin. Thereafter, liver paraffin blocks were cut into 7 µm thickness sections using a microtome (Leica RM 2145) and mounted on glass slides. Sections were stained with hematoxylin-eosin (H&E) for histological evaluation. The histological sections were examined under the Leica DM750 microscope and the images were captured using the Leica ICC 50 HD camera (Germany) connected to the microscope.

Statistical analysis was carried out using the MedCalc Statistical Software version 18 (MedCalc Software byba, Ostend, Belgium; <http://www.medcalc.org>; 2018). Quantitative data were expressed as mean±standard deviation. Differences between measurements were assessed using ANOVA for repeated measures. A p-value <0.05 was considered statistically significant.

Results and discussions

Concentration of berberine in BVE

The berberine content in the methanolic *Berberidis cortex* extract was of $30.4\pm 0.01\%$ (w/w).

The effect of BVE on lipid profile

A diet based on lard induced a significant increase in both serum triglycerides (TG) and total cholesterol (TC) in rats. As expected, in the control group there were no significant changes in both TC and TG. All animals treated with BVE or atorvastatin had total cholesterol lower compared to both control and group II treated with saline solution. The reduction of lipid parameters is similar in experimental groups, treated with different concentrations of BE or atorvastatin with no statistical significance among them (table1).

The effect of BVE on liver function

AST, ALT mean values measured before treatment and after treatment can be found in table 2. Variations in AST and ALT level measurements were neither different between the two determinations nor between groups (table 2).

Changes in liver morphology

The control group presents a normal hepatic architecture, with classic hepatic lobules containing hepatocytes with a normal histological aspect. Also, portal spaces have normal histology. Compared with the control group, examination of liver sections in group II shows several histological changes in the hepatic structure. In the rat liver of this group, some ballooned hepatocytes with intracytoplasmic lipid droplets and vacuolated nuclei (glycogenated nuclei) may be observed along with a

moderate inflammatory infiltration of the portal space. Despite these changes, fibrosis is not observed. The general aspect suggests moderate non-alcoholic steatohepatitis. In the third group, a moderate inflammatory infiltrate of lymphocytes and some plasma cells is found in the portal spaces. The overall appearance of hepatocytes has not been altered. Hepatic histology in rats from groups IV and V is similar. An important inflammatory infiltrate can be seen in the perilobular and intralobular areas. Radial strings of hepatocytes have almost normal architecture in these groups (fig. 1).

The effect of BVE on renal function

Also, renal function parameters (urea and creatinine) were determined. Variations in urea level measurements were neither different between the two determinations nor between groups. Variations of creatinine levels between determinations were found (table 3).

Table 1

EFFECT OF BVE ON PLASMA LIPIDS IN HIGH FAT DIET INDUCED HYPERLIPIDEMIC RATS. ALL THE VALUES ARE EXPRESSED AS MEAN±SD

Treatment group	1 st determination		2 nd determination		p values TC	p values TG
	TC	TG	TC	TG		
I	72.5±19.3	72.9±36.7	71.2±18.8	68.5±80.4	0.001	<0.001
II	72.1±22.6	88±21.9	60.4±12.6	104.9±55		
III	100.1±37.6	148.2±91.1	46.6±12.5	28±9.7		
IV	120.4±34.1	246.6±80.1	41±10.1	32.2±7.8		
V	72.6±21.6	111.8±74.7	33.4±10.2	22.5±5.1		

Table 2

EFFECT OF BVE ON AST AND ALT IN HIGH FAT DIET INDUCED HYPERLIPIDEMIC RATS. ALL THE VALUES ARE EXPRESSED AS MEAN±SD

Treatment group	1 st determination		2 nd determination		p values AST	P values ALT
	AST	ALT	AST	ALT		
I	112.5±34	96.5±21.7	114.8±43	95.1±18.9	0.3	0.6
II	153.4±55.9	56.9±18.5	316±109.4	72.1±15.6		
III	111±48.1	59.5±9.4	307.8±163.2	68.2±22.9		
IV	94.1±40.7	79.5±30.3	320.5±81.9	92.8±47.7		
V	361.9±125.2	80.4±26.7	288.6±95.2	78.5±17.5		

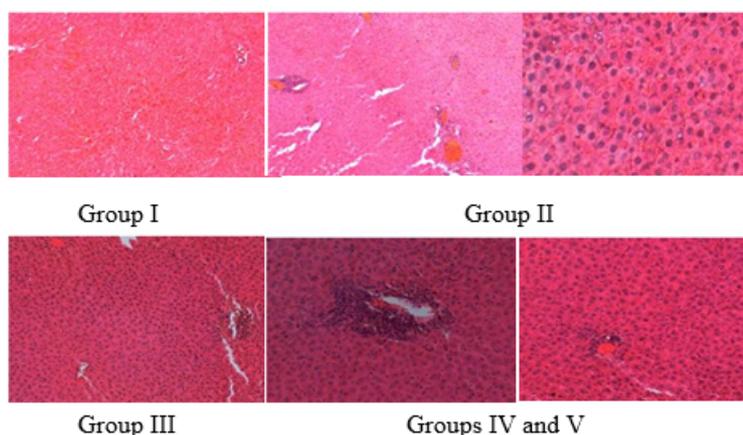


Fig. 1. Effect of BVE on histological profile of liver

Table 3

EFFECT OF BVE ON UREA AND CREATININE IN HIGH FAT DIET INDUCED HYPERLIPIDEMIC RATS. ALL THE VALUES ARE EXPRESSED AS MEAN±SD

Treatment group	1 st determination		2 nd determination		p values urea	p values creatinine
	Urea	Creatinine	Urea	Creatinine		
I	362.2±61.8	1.0±0.1	376.6±68	0.8±0.1	0.1	<0.001
II	294.9±108.7	1.1±0.2	341.4±96	1±0.2		
III	363.3±120.4	1.2±0.1	247.5±76.1	0.9±0.1		
IV	424.7±29.5	1.2±0.1	266.1±44.5	0.7±0.1		
V	443.9±154.8	1.2±0.1	226.7±82.6	0.7±0.2		

The present study investigated the effect of BVE on lipid parameters, on kidney tests and on non-alcoholic fatty liver changes due to lard-based high fat diet. BVE reduced serum lipids similar to atorvastatin, without affecting liver or kidney function, in both used concentration.

The ethanolic extracts used in this experiments had the berberine concentrations of 90mg/kg (group III) and 152mg/kg (group IV), respectively. The concentration of berberine in the extract was similar to the concentration of berberine used as pure standard as reported in other studies (e.g. 75 mg, 100mg, 120mg, 200mg) [19–22].

Cardiovascular disease and non-alcoholic fatty liver disease (NAFLD) are important vulnerable conditions that contribute to morbidity and mortality, especially in developing countries due to inadequate lifestyle and nutritional changes. In Romania, many people suffer from cardiovascular problems or NAFLD, and the first involved factor is dyslipidemia due to an unhealthy diet based on animal fats, especially lard [23, 24]. As a particularity, in this study we used bark extract of *Berberis vulgaris* from Transylvania, Romania; dyslipidemia has been induced with lard, not with standard high fat diet. As expected, due to an unhealthy diet rich in lipids, we could determine an increase in lipid parameters and changes in liver structure similar to NAFLD. These changes were significant, especially in the second group.

We have shown that BVE has succeeded in improving the lipid profile of serum. Oral administration of BVE in rats for one month reduced total cholesterol by 53.4% in IIIrd group, by 65.94% in IVth group and by 53.99% in Vth group. The BVE was efficient in both administered concentrations and the efficacy was similar with that of atorvastatin. However, it can be seen that the percentage reduction in TC is higher in the group treated with high dose of BVE than in the group treated with atorvastatin.

Our findings are in agreement with the earlier reports. There are many *in vivo/in vitro* studies which demonstrated that berberine decreases the blood lipids (cholesterol, triglyceride) [25, 26] but in this study, we investigated whether BVE can achieve the hypolipidemic effect of statins.

In the previously published data, the effect on lipid profile was investigated. In Li et al., [27] study berberine was administered in a dose of 150 mg/kg/day for 4 weeks orally. The results showed a significant decrease of TG and TC, similar to our study. But, in the present study, we used the extract of *Berberis vulgaris* instead of berberine as a pure substance.

In the present study, we did not investigate the mechanism of berberine in reducing cholesterol level. Inhibition of intestinal absorption, intraluminal micelisation of cholesterol, decreased absorption and enterocytic cholesterol secretion are considered to be the main mechanisms of reduced cholesterol [28].

Wong et al. [29] have shown that berberine significantly inhibits intestinal absorption of cholesterol. As a result, plasma cholesterol decreases. It has also been shown that berberine increases the excretion of cholesterol from the liver in the bile and then in the feces [26]. Furthermore, berberine may activate the extracellular signal-regulated kinase pathway and thus regulates the expression of the LDLR gene [25].

Even though cholesterol is an important structural compound of the biological membrane and is the precursor to many other essential organic compounds (steroid hormones, vitamin D), high serum cholesterol increases the risk of cardiovascular disease [30]. The liver has an

important role in the transport, metabolism, and excretion of cholesterol [4].

So, it was reasonable to study the role of BVE in hyperlipidemic-induced liver disorders. Thus, cells containing excessive lipids were observed in histopathological changes of the liver in rats fed with lard. BVE has improved histological changes in the liver tissue along with the lipid-lowering effect.

Although ALT and AST are important liver enzymes and their serum levels have been established as specific and reliable hepatic impairment biomarkers [31], the beneficial effect was not observed on these in our study because they did not significantly increase, maybe due to the short duration of the hyperlipidemic diet. However, it was observed that both concentrations of BVE improved hepatic necroinflammation secondary to dyslipidemia. In the groups fed with lard, but without hypolipidemic treatment, many changes were observed compared to the other treatment groups. Changes have been established as NAFLD. This is histologically defined by the presence of hepatic steatosis in more than 5% hepatocytes at macrovesicular and/or microvesicular level [32].

In another study, the risk of renal dysfunction was correlated with the increase of TC, HDL-C or a high TC/HDL ratio, especially for those patients with an initial creatinine of less than 1.5 mg/dL [33]. In our study, creatinine values decreased in rats in the BVE or atorvastatin-treated groups. Statins have been shown to improve coronary blood flow. As a consequence, it is possible to increase renal blood flow and, consequently, to decrease serum creatinine [34].

Conclusions

The findings of the current study have suggested that BVE intake suppresses the accumulation of hepatic lipids and lipid parameters (TC and TG) and could reduce the risk of CV diseases and also NAFLD. So, BVE can be useful as a monotherapy or in combination with statins.

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