Communication

Sea Buckthorn and Grape Extract Might Be Helpful and Sustainable Phyto-Resources as Associated Hypolipidemic Agents—Preliminary Study

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Abstract: Phytotherapy can enhance the beneficial health outcomes in the prevention of obesity and is able to improve the function of the metabolic organs, like the liver and kidneys. Since sea buckthorn (SBT) and grape extracts are known as abundant sources of polyphenol, we assumed that the extracts of these two plants might have a hypolipidemic effect and an improved metabolic function in obese rats treated with atorvastatin. One hundred and twelve white Wistar rats were divided equally into seven groups (G.I–VII) and orally treated as follows: G.I, atorvastatin 20 mg × kg·bw^{-1}; G.II, atorvastatin 20 mg × kg·bw^{-1} + SBT 100 mg × kg·bw^{-1}; G.III, atorvastatin 20 mg × kg·bw^{-1} + grape extract 100 mg × kg·bw^{-1}; G.IV, grape extract 100 mg × kg·bw^{-1}; G.V, SBT 100 mg × kg·bw^{-1}; G.VI, high-fat diet (HFD); group VII was considered the control group. After two and six months of administration, the rats were sacrificed, and blood samples were taken for biochemical analyses. The statistical results (analysis of variance (ANOVA)) showed that a combination of SBT and grape extracts with atorvastatin significantly reduced ($p < 0.001$) the lipid parameters. After six months, the liver and kidneys improved their functioning, showing a statistically significant change ($p < 0.001$) in the grape and sea buckthorn groups compared to the other groups. In addition, grape extract and SBT combined with atorvastatin proved to be potent hypolipidemic agents, so associations with phytodietary supplements can be considered as a valuable means of combating hypolipidemia and decreasing risk factors.

Keywords: dyslipidemia; sea buckthorn; grape; high-fat diet; rats; phytotherapy; atorvastatin

1. Introduction

Dyslipidemia is characterized by irregular levels of lipid parameters, such as low-density lipoprotein cholesterol (LDL-*c*), triglycerides, high-density lipoprotein cholesterol (HDL-*c*),

and atherogenic fractions in the bloodstream. It is one of the current abnormalities and is one of the main components of the metabolic syndrome, as well as a frequent challenge that leads to morbidity and mortality [1]. Lipid and lipoprotein irregularities introduce risk factors for cardiovascular diseases, and the predominance of these in the worldwide community has developed considerably in the last decades. Studies have exposed that dyslipidemia is very widespread among males aged 30–40 years, justifying the raised prevalence of infarctions due to coronary artery disease in this age group [2]. Data has shown that lipid-lowering therapies can diminish the growth of coronary atherosclerosis [3]. With this aim, a major goal of clinical treatment of cardiovascular disease (CVD) is risk minimization by completing healing target levels for any lipid parameter [4]; for regulating these, "statins" are involved, since they can reduce the LDL-*c* levels by up to 55% [5,6].

Statins are structures with anti-atherosclerotic characteristics; atorvastatin is considered the first choice of drug for the management of heart disease, as it alters the reversible inhibition of hydroxymethylglutaryl coenzyme A (HMG-CoA), but, like all medications, is also has potential adverse effects (the most known is liver toxicity through elevation of transaminases enzymes) [7].

Along with developments in the occurrence of chronic metabolic diseases, there is a growing interest in new natural resources. Phytomedicine with respect to CVDs has acknowledged many phytochemicals, including carotenoids, isorhamnetin, anthocyanins, resveratrol, etc. These were proven to have potential biological activities both in vivo and in vitro, such as eliminating free radicals, anti-tumor potential, and anti-inflammation potential; In addition, they include some amounts of trace elements, vitamins, and essential fatty acids [8].

Recent lifestyle changes with high-fat diets (HFDs) have resulted in increases in the prevalence of obesity, which, unfortunately, is associated with chronic disease, like fatty liver or type II diabetes. HFDs can cause impairment of metabolic status, including altering transaminases and the lipogenesis pathway, which leads to excessive accumulation of triglyceride (TG) in hepatic tissues, fibrosis, hepatic cellular ballooning, steatosis, or inflammation. Therefore, liver protection is linked to the inhibition of hepatic fat accumulation by restraint of lipolysis and lipogenesis pathways [4].

Recently, there has been a lot of attention on HFD-related kidney damage, and it is now increasingly comprehended as an independent risk factor for chronic kidney disease. HFDs, with their associated cardiovascular disease risk, can also affect the kidney, and show a highly significant increase in the concentration of serum urea, uric acid, and creatinine. HFDs induce alteration of the metabolism of renal lipids due to the inequality between lipogenesis and lipolysis in the kidneys, in addition to the systemic metabolic irregularities; following renal lipid accumulation, it leads to renal injury [9].

Sea buckthorn (SBT) (*Hippophae rhamnoides L.*) is a shrub that belongs to the family *Elaeagnaceae*. All parts of this plant (but especially fruits and leaves) are considered to be a rich source of bioactive substances with many different pharmacological effects on wellbeing, such as anti-atherogenic effects, anti-oxidant effects, anti-cancer effects, etc. [10–12]. Currently, research has focused on the effect of Sea buckthorn or its extracts on cardiovascular disease. Furthermore, outcomes from experimental studies with SBT or its extracts on blood lipid profiles were inconsistent due to differences in experimental models, study plans, and health states, but supplemental SBT certainly enhanced the blood lipid profiles in patients suffering from heart disease [13].

Grape (*Vitis Vinifera*) extract is an important reservoir of polyphenols, mainly tannins, resveratrol, and flavonoids [13,14], polyphenols being among the most common phytochemicals added in diets [15]; they are considered as a powerful health promoter in hyperlipidemia [16].

This study aimed to investigate the hypolipidemic effect of Sea buckthorn and grape extract in rats fed for a prolonged period with HFD in relation to the atorvastatin administration. Consequently, comparative observations were made to assess the effectiveness of phytotherapy with respect to induced hyperlipidemia and the opportunity to associate these phyto-structures as a component of rats' diet alone or in combination with the allopathic hypolipidemic structures.

2. Materials and Methods

2.1. Experimental Animals and Design

To assess the protective influences of phytotherapy with respect to hyperlipidemia caused by a high-fat diet (HFD), 112 white Wistar rats weighing between 150 and 165 grams and with ages of 3–4 months were included in this study. They were purchased from the National Research and Development Institute for Microbiology and Immunology "Cantacuzino" (NIRDMI) (Bucharest, Romania). The rats were housed in regular cages (l × w × h = 750 × 720 × 360 mm) and fed ad libitum with a usual diet (Diet, Biovetimix, code 140-501, Romania). For bedding, wood shavings were used. All animals were put in specific free-animal rooms in a controlled environment at 22 ± 2 °C with a humidity of 55 ± 10% and with 12 h light/12 h dark.

The investigation was approved by the Ethical Committee of the Faculty of Veterinary Medicine of Banat's University of Agricultural Science and Veterinary Medicine in Timișoara under no. 6/30.01.2019. Before the inception of the study, animals were put in cages for seventeen days to acclimatize, and were managed under Directive 2010/63/EU [16] on the management of animals used for scientific goals (Directive 2010) and the guidelines of the National Research Council (NRC). Animals were divided into seven experimental groups, each group comprising 16 animals fed with a known regular HFD [17], except for the control group [18]. Animals were dosed orally for the analyzed biochemical tests, as presented in Table 1.

Table 1. Experimental plan and dosage for all experimental groups.

Group	Animals No.	2 Months	6 Months	Dosage
I	16	8	8	Atorvastatin (*Sortis, Pfizer Europe*)—20 mg × kg·bw^{-1}
II	16	8	8	Atorvastatin + sea buckthorn: 20 mg + 100 mg × kg·bw^{-1}
III	16	8	8	Atorvastatin + Antioxivita: 20 mg + 100 mg × kg·bw^{-1}
IV	16	8	8	Antioxivita (*Phenalex, Romania*)—received 100 mg × kg·bw^{-1}
V	16	8	8	Sea buckthorn—received 100 mg × kg·bw^{-1}
VI	16	8	8	Control (positive)—received high-fat diet (HFD)
VII	16	8	8	Control (negative—received only normal diet (ND)

Legend: kg·bw^{-1}—kilogram/body weight; HFD—high-fat diet; ND—normal diet.

2.2. Atorvastatin

Atorvastatin *(Sortis, Pfizer Europe)* belongs chemically to the statin group, with a molecular formula $C_{33}H_{35}FN_2O_5$ and molecular weight 558.65 g/mol; this structure belongs to the group named diphenylpyrrols, as it comprises heterocyclic aromatic molecules with a pyrrole ring attached to two phenyl groups. Atorvastatin is a 3-hydroxy-3-methylglutary CoA (HMG-CoA) reductase inhibitor, acting by lowering LDL cholesterol in hyperlipidemia. It was obtained from Help Net pharmacy, Timișoara, Romania. It was used as an oral suspension in distilled water at a dose level of 20 mg × kg·bw^{-1}, which is considered the lowest therapeutic dose level in humans [19].

2.3. Plant Material and Preparation of Sea Buckthorn Extract

Sea buckthorn fruits were purchased from a natural plant store. The method of extraction was arranged to receive 100 mg polyphenol for each administered dose [20]. Ten grams of fresh fruits were crushed using a laboratory grinder, then weighed and added to 100 mL ethanol 70%, put in a shaker for one hour after that filtered, and transferred to the rotary evaporator at 70 °C until a final solution was obtained. The total polyphenol amount was measured according to the Folin–Ciocalteu method. The method was replicated many times to obtain the total amount of extract necessary for the whole research period [21]. Chemical compounds of total polyphenol obtained from Sea buckthorn extract

were measured using HPLC (Table 2). The dose of SBT was calculated such that 10 g of SBT included 26 mg of polyphenol, and 30 mL of the concentrated extract included 624 mg of polyphenol. So, using the rule of three, the given dose was 100 mg $\times$ kg·bw^{-1}, which indicates 1.5 mL of SBT extract/rat.

Table 2. Individual determinations for total polyphenol in the sea buckthorn extract used ($\mu g \times mL^{-1}$).

No.	Compound Name	Retention Time	Area	Height	Concentration
1	Gallic acid	0.000	0	0	0.000
2	Protocatechuic acid	0.000	0	0	0.000
3	Caffeic acid	0.000	0	0	0.000
4	Epicatechin acid	0.000	0	0	0.000
5	*p*-coumaric acid	0.000	0	0	0.000
6	Ferulic acid	0.000	0	0	0.000
7	Rutin	0.000	0	0	0.000
8	Rosmarinic acid	28.851	4,542,389.0	116,917.0	43.742
9	Resveratrol	30.235	4,511,738.0	86,437.0	28.385
10	Quercetin	32.087	2,426,515.0	77,684.0	40.534
11	Kaempferol	33.636	109,323.0	4451	6.208

2.4. Antioxivita

Antioxivita *(Phenalex, Romania)* was bought from a local herbal pharmacy; it is formed of water, seed extract, skin, and grapeseed. The total polyphenol content in this product included: 300 mg $\times$ mL^{-1} gallic acid equivalent (GAE), phenolic acids, anthocyanins, flavonoids, tannins, catechins, and resveratrol (Table 3).

Table 3. Individual determinations for total polyphenols in the grape extract used ($\mu g \times mL^{-1}$).

No.	Compound Name	Retention Time	Area	Height	Concentration
1	Gallic acid	0.000	0	0	0.000
2	Protocatechuic acid	0.000	0	0	0.000
3	Caffeic acid	0.000	0	0	0.000
4	Epicatechin acid	0.000	0	0	0.000
5	*p*-coumaric acid	24.945	86,367.0	3501	0.101
6	Ferulic acid	0.000	0	0	0.000
7	Rutin	26.522	997,509.0	24,804.0	7.525
8	Rosmarinic acid	28.466	2,728,108.0	71,428.0	26.271
9	Resveratrol	29.154	2,958,778.0	68,286.0	18.615
10	Quercetin	30.890	12,200,171.0	87,510.0	203.798
11	Kaepferol	34.636	4,764,393.0	36,177.0	270.556

2.5. Clinical Biochemistry Analyses

The concentrations of triglyceride (TG), serum total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), urea, uric acid, and creatinine were measured on a Randox Daytona Plus (Randox, UK) automatic biochemistry analyzer using the corresponding commercial kits (Randox, UK). The calibration was performed with Randox Calibration Serum Level 3 (Cat. No. CAL 2351), and the QC charts for reference materials were created using two levels of control serum: Randox Assayed Multisera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532), while VLDL was measured by divided triglyceride/5.

2.6. Statistical Analysis

The statistical software used was Graph Pad Prism 6.0 for Windows (Graph Pad Software, San Diego, USA). Values were expressed as mean ± SEM (standard error of means). The estimation of the difference between groups was ascertained using two-way analysis of variance (ANOVA) with Tukey's multiple comparison tests. The statistical values were considered as follows: * $0.01 \leq p < 0.05$, significant; ** $0.001 \leq p < 0.01$, highly significant; *** $p < 0.001$, very high significant; [ns]: not significant.

3. Results

In the present study, phytotherapy was shown to possess a good hypolipidemic and hepatoprotective activity. The treatment with 100 mg × kg·bw^{-1} of both Sea buckthorn and grape extract in combination with the atorvastatin showed a statistically significant reduction in the lipid and liver parameters, especially after six months of observation.

The first sign of hyperlipidemia was the registered values for weight gain, as well as, biochemically, abnormalities in triglyceride (TG) and total cholesterol (TC), so they are investigated in this study.

In Table 4, the weight evolutions of the studied groups (divided according to sex) after two and six months of observation are presented.

Table 4. The weight evolutions of the studied groups at two and six months of observation.

Group	Average Weight of the Rats after:			
	Two Months		Six Months	
	Female	Male	Female	Male
I	265.2	473.5	329.2	496.3
II	252.7	458.2	319.0	463.0
III	241.0	414.7	314.3	425.0
IV	266.0	502.7	325.2	553.2
V	286.5	507.0	360.7	563.2
VI	360.0	522.0	378.0	606.0
VII	312.7	499.0	340.7	552.0

As shown in Table 5, the TC and TG levels of rats in the group fed with an HFD was significantly higher than those of the control group ($p < 0.001$), meaning that the TG levels of rats in the control group showed a favorable tendency after being fed with an HFD. Compared with the control, the mean values of the lipid parameters in the experimental groups I, II, III, IV, and V, which were analyzed at the baseline and measured for two months, showed no statistical differences after two months of treatment, while after six months, the results were totally different; the mean values of the TC, TG, and LDL-*c* in all these groups significantly decreased. The HFD group illustrated a significant rise in TC (95.9 ± 5.1), TG (245.6 ± 30.1), and LDL-*c* (16.2 ± 1.6) as well as a clear decline in HDL-*c* (7.6 ± 0.9) after six months of HFD administration.

Significant alterations were observed in all lipid parameters in the groups with atorvastatin + SBT and atorvastatin + grape extract that received these combinations for six months, as shown in Table 5.

In this study, we ascertained that phytotherapy was proficient in the alteration of liver enzymes in the rats' serum. It was observed that the rats that got grape extract and sea buckthorn alone revealed low values of serum enzymes when compared to the atorvastatin and HFD groups ($p < 0.001$). The liver enzyme levels in atorvastatin- and HFD-fed rats were significantly increased (ALT - 65.5 ± 12.4; AST = 286.3 ± 94.0; AP = 524.04 ± 72.1 = $p < 0.001$), while for HFD, the statistics were ALT = 62.3 ± 6.7; AST = 159.5 ± 21.5; AP = 488.5 ± 56.4 = $p < 0.001$, with respect to rats fed with a normal diet (Table 6).

As shown in Table 7, there were no statistical differences after two months of treatment. However, a significant difference was observed in urea after six months in the atorvastatin group ($n = 8$) (55.7 ± 19.6) and HFD group ($n = 8$) (43.6 ± 2.7) compared with other groups.

Table 5. Lipid profile parameters in the experimental groups after two and six months of phytotherapy.

Group	Mean (mg/dL) ± SE									
	Cholesterol		Triglyceride		HDL-*c*		LDL-*c*		VLDL	
	2 Months	6 Months	2 Months	6 Months	2 Months	6 Months	2 Months	6 Months	2 Months	6 Months
I	85.6 ± 5.3 *	77.8 ± 10.4 ^{ns}	166.6 ± 22.2 **	91.8 ± 21.4 ^{ns}	19.6 ± 0.7 ^{ns}	26.7 ± 1.8 ^{ns}	9.6 ± 1.8 ^{ns}	7.3 ± 0.1 ^{ns}	33.3 ± 4.4 **	18.3 ± 4.1 ^{ns}
II	83.9 ± 3.5 *	74.8 ± 7.2 ^{ns}	158.2 ± 32.8 **	86.9 ± 16.8 ^{ns}	20.6 ± 2.2 ^{ns}	28.1 ± 1.6 *	8.6 ± 1.2 ^{ns}	7.1 ± 0.4 ^{ns}	31.6 ± 6.5 **	17.3 ± 3.3 ^{ns}
III	73.5 ± 6.4 ^{ns}	57.6 ± 7.6 ^{ns}	119.8 ± 9.5	84 ± 16.7 ^{ns}	21.1 ± 2 ^{ns}	32.7 ± 3.4 ***	9.1 ± 1.1 ^{ns}	6.9 ± 0.4 ^{ns}	23.9 ± 1.8 ^{ns}	16.8 ± 3.3 ^{ns}
IV	93.5 ± 4.8 **	80 ± 9.4 ^{ns}	169.1 ± 26.8 **	107 ± 12.8 ^{ns}	18.1 ± 1 ^{ns}	26.2 ± 2.3 *	11.1 ± 1.3 *	7.4 ± 0.5 ^{ns}	33.8 ± 5.3 **	21.4 ± 2.5 ^{ns}
V	93.8 ± 2.8 **	79.4 ± 11 ^{ns}	176.2 ± 44.4 **	143.4 ± 24.1 ^{ns}	18.5 ± 0.6 ^{ns}	22.8 ± 1.6 ^{ns}	11.2 ± 1.4 *	8.5 ± 0.3 ^{ns}	35.2 ± 8.8 ***	28.6 ± 4.8 ^{ns}
VI	88.2 ± 7.0 **	95.9 ± 5.1 ^{*ns}	212.3 ± 27.3 ***	245.6 ± 30.0 ^{***ns}	9.3 ± 0.8 ^{ns}	7.6 ± 0.9 ^{***ns}	13.7 ± 2.4 ***	16.2 ± 1.6 ^{***ns}	42.4 ± 5.4 ***	49.1 ± 6 ^{***ns}
VII	46.9 ± 13.9	53.5 ± 5.9	33.5 ± 7.5	52.7 ± 7.5	24.7 ± 1.5 ***	25.7 ± 2.8 ^{ns}	4.9 ± 0.7	6.8 ± 0.4 ^{ns}	6.7 ± 1.5	10.5 ± 1.5 ^{ns}

Comparison with control: ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Comparison of two months vs. six months: ^{ns} $p > 0.05$.

Table 6. Liver enzyme activity in the experimental groups after two and six months of treatment.

Group	Mean (U/L) ± SE					
	ALT		AST		AP	
	2 Months	6 Months	2 Months	6 Months	2 Months	6 Months
I	41.1 ± 5.7 ^{ns}	65.5 ± 12.4 ***	171.5 ± 39 ^{ns}	286.3 ± 94 ^{***ns}	469.3 ± 84.5 ^{***ns}	524 ± 72.1 ^{***ns}
II	41.8 ± 11.6 ^{ns}	38.7 ± 6.5 ^{ns}	157.9 ± 15.1 ^{ns}	120.8 ± 7.4 ^{ns}	333.1 ± 38.7 ^{ns}	216.3 ± 23.6 ^{ns}
III	37.3 ± 6.2 ^{ns}	27.4 ± 3.0 ^{ns}	129.8 ± 21 ^{ns}	106.2 ± 7.9 ^{ns}	197.2 ± 38.2 ^{ns}	179.9 ± 39 ^{ns}
IV	27.8 ± 1.3 ^{ns}	25.4 ± 2.6 ^{ns}	100.6 ± 7.7 ^{ns}	90.8 ± 7.3 ^{ns}	167.9 ± 34.7 ^{ns}	167.7 ± 30.6 ^{ns}
V	31.1 ± 3.9 ^{ns}	27.3 ± 2.6 ^{ns}	115 ± 16.5 ^{ns}	95.8 ± 4.6 ^{ns}	177.3 ± 22.9 ^{ns}	174.7 ± 19.5 ^{ns}
VI	39.2 ± 4.5 ^{ns}	62.3 ± 6.7 ^{***ns}	138.6 ± 27.3 ^{ns}	159.5 ± 21.5 ^{ns}	395.8 ± 35.7 ^{**ns}	488.5 ± 56.4 ^{***ns}
VII	20.3 ± 1.6	24.8 ± 1.6	79.6 ± 4.6	95.2 ± 6.9	161.9 ± 33.6	169.6 ± 41.7

Comparison with control: ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Comparison of two months vs. six months: ^{ns} $p > 0.05$.

Table 7. Kidney function tests in experimental groups after two and six months of treatment.

Group	Mean (mg/dL) ± SE					
	Urea		Uric Acid		Creatinine	
	2 Months	6 Months	2 Months	6 Months	2 Months	6 Months
I	54.5 ± 15 *	55.7 ± 19.6 *	1.8 ± 0.0 ns	2.1 ± 0.636 **	0.6 ± 0.0 ***	0.7 ± 0.0 ***
II	43.3 ± 1.8 ns	28.6 ± 0.7 ns	1.4 ± 0.0 ns	0.9 ± 0.059 ns	0.7 ± 0.0 ***	0.6 ± 0.0
III	42.3 ± 2.8 ns	29 ± 0.4 ns	1.4 ± 0.2 ns	0.9 ± 0.1 ns	0.7 ± 0.0 ***	0.6 ± 0.0
IV	26.3 ± 1.8 ns	23.6 ± 1 ns	1 ± 0.1 ns	0.9 ± 0.0 ns	0.6 ± 0.0	0.6 ± 0.0
V	35 ± 3.2 ns	26.7 ± 1.8 ns	1.1 ± 0.0 ns	0.7 ± 0.1 ns	0.7 ± 0.0 ***	0.6 ± 0.0
VI	38.6 ± 2.1 ns	43.6 ± 2.7 ns	1.3 ± 0.2 ns	1.7 ± 0.3 ns	0.6 ± 0.0 *	0.7 ± 0.0 ***
VII	23.3 ± 1.2	24.4 ± 1.2	0.7 ± 0.1	0.9 ± 0.0	0.5 ± 0.0	0.5 ± 0.0

Comparison with control: ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Comparison of two months vs. six months: ns $p > 0.05$.

4. Discussion

Due to their relationship with atherogenic dyslipidemia, obesity and metabolic syndrome are linked with an enhanced danger of CVD [21,22].

Dyslipidemias comprise a wide-ranging variety of lipid abnormalities, some of which are of great importance in CVD. High levels of TC and LDL-*c* have been determined to be of main importance, mostly because they can be tailored by treatments. Randomized controlled trials (RCTs) have proven that reducing TC and LDL-*c* can prevent CVD; therefore, TC and LDL-*c* are the primary targets of therapy [23].

In addition, some other types of dyslipidemias emerge that cause predisposition to early CVD. Among these, the co-existence of augmented VLDL residues (mirrored by slight TG presence), increased LDL-*c* elements, and low HDL-*c* levels—recognized as the "lipid triad"—is frequently considered as major target of CVD prevention [23].

Our outcomes demonstrated a highly significant reduction in all lipid values in the atorvastatin + SBT and atorvastatin + grape extract groups when compared to the HFD and control groups. These reductions indicate that the added phytotherapy could improve the process of managing hyperlipidemia.

Another notable result was an enhancement in HDL-*c* level, which is considered the good cholesterol, since it has a protective influence against heart disease. Atorvastatin significantly decreased lipid values in the atorvastatin group due to its inhibition of HMG-CoA reductase in the microsomal cells, as well as its alteration of the serum LDL-*c* by improving LDL-receptor-mediated LDL uptake [24].

The present study demonstrated that the addition of 100 mg × kg·bw^{-1} of sea buckthorn in combination with atorvastatin, or only as a dietary supplement in obese rats, could reduce the serum TC and increase HDL-*c*. These findings were in agreement with those in a previous study done on the hamsters, which demonstrated that SBT seed oil could effectively reduce plasma LDL-*c* and exhibited anti-atherogenic activity [25]. This study supports our observations that hyperlipidemia emerges from long-term irregularities in lipid metabolism, and reducing serum lipid levels through dietary treatment alone or with drug treatment appears to be associated with a certain decrease in the risk of vascular disease and the related complications [25].

The hypocholesterolemic and LDL-*c* lowering properties of SBT extract were observed in this study and could be valuable information for the prevention of CVD through the improvement of dyslipidemia [26]. The SBT-supplemented rats demonstrated a remarkable lowering in the lipid parameters, which might be partially mediated by inhibition of cholesterol synthesis. Furthermore, polyphenols and polyunsaturated fatty acids can decrease cholesterol by suppressing hepatic cholesterol synthesis, as other authors also stated [27,28].

In addition to that, from the point of view of nutritional value, SBT contains a large amount of chemical structures that are valuable for health, including kaempferol, phenolics, quercetin, iso-rhamnetin, fat-soluble vitamins (A, K, and E), soluble vitamins (C, B1, B2, and B11), carotenoids, and other helpful nutrients [29], which have certain hypolipidemic properties.

Therapy with grape extract demonstrated a significant decrease in the lipid values induced by HFD in rats due to inhibition of lipogenesis and VLDL in the liver, which were described as results of the HFD. Therefore, consumption of proanthocyanin- and polyphenol-rich foods (such as grapes) can manage atherogenic dyslipidemias associated with obesity [30]. These results are in agreement with those of Del Bas et al. [31].

The literature reported that an HFD is one of the main constituents that causes obesity in human and animal models [32]; in addition to causing obesity and excessive intake of fat, it also raises the state of co-morbidities, such as hypertension, hyperlipidemia, CVD, and diabetes mellitus [33]. Accordingly, the consumption of phyto-compounds supports the physiological balance of the redox status, since these natural sources have many antioxidant compounds capable of fighting oxidative stress [34].

Abnormal hepatic transaminase levels are generally identified as a result of atorvastatin therapy. In our case, it was certain that the transaminases increased within the experimental period. Our trial revealed significantly elevated ALT and AST values—three times the normal limit, which is considered a toxic endpoint. The present study assessed the hepatoprotective potential of the continuous use of grape extract on aminotransferase enzymes in the livers of obese rats. Grapes have been studied extensively because of their antioxidant properties [35]. Now, it is well described in the literature that grape extract can offer a good protection against neurodegenerative and metabolic disease [36]. *Vitis vinifera* ethanol extract was also found to have a significant protective effect by reducing the serum levels of AP and total bilirubin [37].

The current study also proved that rats fed with an HFD for a six-month period produced a change in the aminotransferase activity. In addition, ALT—a marker for hepatic injury—was significantly raised in the HFD group (62.3 ± 6.7) in comparison with the control (24.8 ± 1.6). This elevation was due to the free radical injury that induced the oxidative process, which is considered as a process responsible for the change toward inflammation, fibrosis, and hepatocellular damage [38].

However, both the 100 mg $\times$ kg·bw^{-1} grape extract alone (25.4 ± 2.6) and 100 mg $\times$ kg·bw^{-1} SBT in SBT (27.3 ± 2.6) supplementation evidently improved the ALT activities. This research also suggested that the presence of polyphenols (and phytotherapy supplementation) may reduce hepatic injury by enhancing the antioxidant defense system and by controlling the aminotransferases' value. The presented data are in line with previous studies explaining that mice fed with an HFD and grape phytochemicals had a significant reduction in hepatic aminotransferase in obese animals [39].

Dani et al. [34] observed that purple grape juice is antitoxic, preventing the damage caused by carbon tetrachloride (CCL4) in the rats' livers. This prevention was attributed to the rich polyphenol content of the grape. Our study confirmed that with HFD-induced hepatotoxicity through elevated aminotransferase enzymes, grape extract combined with atorvastatin therapy reduced the liver damage, proving the grape's effective protection.

Kidneys are dynamic organs that serve as major systems for maintaining the body's homeostasis; they are influenced by various chemicals and drug structures. By evaluating the kidney biochemical parameters, our study also investigated the potential reno-protective outcome of SBT and grape extract against renal damage caused over time by atorvastatin administration in obese rats. Our results showed that there was no statistically significant difference with a dose of 20 mg $\times$ kg·bw^{-1} of atorvastatin in uric acid and creatinine values during the whole experimental period. There are authors who found that a four-time atorvastatin dose (80 mg $\times$ kg·bw^{-1}) lowered the blood urea nitrogen and creatinine levels in rats [40,41]. In our case, the mixture of 100 mg $\times$ kg·bw^{-1} grape extract associated with 20 mg $\times$ kg·bw^{-1} atorvastatin had the most positive result on the renal function values after six months of therapy compared to the HFD group.

The obese rats from the HFD group had a large significant increase in urea, uric acid, and creatinine values in comparison with the control group, which is in agreement with the results of Cindik et al. [40] and Amin and Nagy [42].

This change can be attributed to the effect of the HFD on the renal lipid metabolism due to irregularity between lipogenesis and lipolysis in the kidney, as well as systemic metabolism variations, leading to renal lipid mass and to renal injury, as other authors observed [43]. In addition, activation of the rennin–angiotensin system, glomerular hyperfiltration, and kidney structural change may lead to more severe glomerular injury, which is linked with continued obesity, a fact that was observed especially after six months, and that is confirmed by other studies [44]; however, this is in disagreement with another study performed on HFD rats treated with grape juice for three months [45].

Additionally the grape extract has been reported to have a broad variety of therapeutic effects, such as anti-inflammatory effects and decreasing apoptotic cell death. The grape extract is known to have high antioxidant activity and to contain different polyphenols, with real demonstrated effects against the vascular damage. These are also associated with free radical scavenging and with anti-mutagenic activity [46]. Grape seed oil and extract have a variety of biologically active classes used for protection, including a multiplicity of biologically active forms used for protection from oxidative stress induced by free radicals and reactive oxygen species (ROS), which are capable of supporting oxidative stress both in vitro and in vivo [47,48].

Fewer studies have explained the effects of SBT on kidney function. From our point of view, the presence of valuable chemicals, nutrients, and numerous biologically active substances, especially rosmarinic acid, resveratrol, quercetin, and kaempferol, explains the high hepatoprotective effect of this plant.

5. Conclusions

The results proved that plants rich in polyphenolic compounds are able to successfully support the metabolic organs' functioning, and that the constant intake of SBT or grape extract—alone or in combination with atorvastatin therapy—may be considered as an adjuvant in the treatment of patients suffering from metabolic-syndrome-like obesity.

SBT and grape extract were shown to be valuable means to reduce injuries produced by the lipid peroxidation in combination with long-term atorvastatin administration, which was attributed to the flavonoid glycosides, including quercetin, kaempferol, resveratrol, and rosmarinic acid. So, phytotherapy can improve biochemical parameters, and it thus proved to be valuable in the preservation of metabolic organs like the liver and kidneys. In our study, rats fed an HFD gained more weight than those that were provided a normal diet. However, the rats fed an HFD along with phytotherapy did not achieve any significant weight gain (Table 4); this indicated that SBT and grape extracts were effective in controlling weight gain.

The supplementation of phytotherapy significantly improved hyperlipidemia, as evidenced by the reduced level of serum total cholesterol and triglyceride as well as the improved HDL-c after six months of therapy (Table 5). This finding is supported by a previous study by Arai et al [49]. There is an inverse link between quercetin intake and TC and LDL-c concentration in Japanese women due to the reduction in hepatic lipogenesis by quercetin [50].

The excessive consumption of fat leads to a number of conditions of damage, mainly in the liver, thus inducing a loss of metabolic processes in the organ and releasing liver enzymes into the blood [51]. In our study (in Table 6), we observed that the HFD provoked an increase in liver enzymes, which is in agreement with a study that analyzed ten weeks of HFD in mice [52]. This could be related to the accumulation of liver lipids, which could cause damage to cellular homeostasis, leading to cytotoxicity [52].

The grape extract was able to prevent increases in AP, ALT, and AST, which was attributed to the polyphenol content; this is in line with another work that also observed that resveratrol was able to attenuate hepatic steatosis in mice [34].

In summary, our data demonstrated that SBT and grape extracts were beneficial in decreasing biochemical parameters and were effective in producing hypolipidemia effects in rats, including controlling body weight and with improving hepatic enzymes; thus, they may have potential as phytochemicals for managing hyperlipidemia.

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