ijlpr 2023; doi 10.22376/ijlpr.2023.13.3.P84-P90

International Journal of Life science and Pharma Research

ISSN 2250-0480

The Influence of Georgian Saperavi Grapevine Shoots Extract on Lipid Profile in Diet-Induced Hypercholesterolemia in Rats

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Abstract: Hypercholesterolemia raises the risk of cardiovascular diseases (CVD), the leading cause of morbidity and mortality worldwide. Numerous clinical trials of lipid-lowering diets and medicines have been established for proper lipid profile regulation to reduce the significant level of pathological conditions of cardiovascular origin. Winemaking is a well-developed part of Georgian agriculture. Besides usual products such as grapes and wine, it is characterized by a significant amount of waste products like leaves and shoots, which can be used as a source of bio-active ingredients, namely polyphenols, which are well-known to have hypocholesterolemia effects. Therefore, the decision was made to use polyphenols derived from wasted shoots and assess their potential protective and treating effect on lipid profile in the Vivo model of dietary-induced hyperlipidemia. During previous studies, 14 individual polyphenols were extracted and detected from Georgian grapevine shoots, and current research demonstrates the cholesterol-lowering effects of this polyphenol-rich extract in laboratory rats. Study results demonstrated the positive protective effect of Georgian *Vitis Vinifera* (Saperavi) polyphenols on the lipid profile of rats. Along with relatively low values of total cholesterol (5.47%), LDL (16%), and TG (9.9%), better results for HDL value were observed for the group receiving Saperavi shoots extract, than the group receiving only a hypercholesterolemia diet. Compared with a 10 mg/kg Atorvastatin daily dose, Saperavi shoots extract showed a smoother reduction of serum cholesterol; however, the normal range was reached. These findings support the potential of using currently wasted Georgian grapevine shoots as a source of biologically active ingredients. Based on the study results, Saperavi shoot extract can be considered a cost-effective candidate for phytomedicine supplementing and treating cardiovascular diseases associated with lipid profile disorders.

Key words: Hypercholesterolemia; Saperavi shoots extract; Waste products utilization; Polyphenols; Phytomedicine

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Received On **11 October, 2022**
Revised On **2 February, 2023**
Accepted On **22 February, 2023**
Published On **1 May, 2023**

Funding This work is supported by the Shota Rustaveli National Science Foundation (SRNSFG) (Ref. number PHDF-21-1607).

Citation Natia Bokuchava, Tamaz Murtazashvili, Nodar Mitagvaria, Koba Sivsivadze and Mariam Tatanashvili , The Influence of Georgian Saperavi Grapevine Shoots Extract on Lipid Profile in Diet-Induced Hypercholesterolemia in Rats.(2023).Int. J. Life Sci. Pharma Res.13(3), P84-P90 http://dx.doi.org/10.22376/ijlpr.2023.13.3.P84-P90

1. INTRODUCTION

Cardiovascular health conditions contribute to the greater part of non-communicable diseases (NCD) mortal outcomes (17.9 million per year), followed by cancer, respiratory diseases, etc. [1]. Hypercholesterolemia raises the risk of cardiovascular diseases (CVD), which is the leading cause of morbidity and mortality[2,3]. Hypercholesterolemia is linked with the increased value of total cholesterol and low-density lipoproteins (LDL) in the blood, as well as with the increased level of triglycerides (TG) and decreased amount of high-density lipoproteins (HDL), which is already established to be a protective factor in the development of atherosclerosis[4,5,6]. Since lipid profile disbalance is one of the biggest issues for the time being, numerous clinical trials of lipid-lowering diets and medicines have been established for lipid profile proper regulation to reduce the significant level of pathological conditions of cardiovascular origin[7-10]. Based on the evidence, the development of HMG-CoA reductase inhibitors (statins) was a big step forward in treating hypercholesterolemia; however, this group of drugs is characterized by substantial side effects, which can be an important barrier to their administration in certain conditions[11-13]. Therefore, the significance of medicinal plants research increases rapidly due to their possible contribution to the various disorders treatment. They can provide diverse pharmacologic effects correlating with their structural specificities. Furthermore, due to the high concentrations of phytochemicals and minerals, herbal medicines have both a medicinal and nutritional impact[14]. Winemaking is a well-developed part of Georgian agriculture. This country is recognized as one of the oldest wine regions, as the earliest evidence of winemaking dates back to 8000 years[15]. Over 500 grape species are grown on the territories of Georgia due to the diverse climate. Besides usual products like grapes and wine, winemaking is characterized by many waste products such as leaves and shoots. The modern approach to waste products considers their utilization for recovering different biologically active ingredients, as this source is cheap and convenient. Georgian grapevine species are constantly in sight of researchers; however, they focus more on wine[16,17], while waste products still need to be explored. Tbilisi State Medical University in collaboration with it. Beritashvili Center of Experimental Biomedicine started to study Georgian grapevine shoots to use derived natural ingredients for pharmaceutical and cosmetic purposes. One of the most interesting bioactive ingredients in Georgian grapevine shoots is polyphenols, which are well-known to have a hypocholesterolemia effect[18-20]. During previous studies, 14 individual polyphenols were extracted and detected from Georgian grapevine shoots: Caffeic acid, Ferulic acid, Isorhamnetine, Kaempferol, Myricetin, P-coumaric acid, Protocatechuic acid, Querc3-O-glu, Quercetine, Resveratrol, Rosmarinic acid, Rutin, Syringic acid, Vanillic acid. Chemical structures for some of them are presented in figure 1. The objective of the current study was to evaluate the potential protective and treating effects of Saperavi grapevine shoots extracted *In Vivo* model of dietary-induced hyperlipidemia. Corresponding studies were conducted on various plants to enclose natural remedies to treat different diseases and make these medications safer and pharmacoeconomic[21,22]. The present research was planned to prove the possibility of using massively wasted Georgian grapevine shoots to prevent and treat cardiovascular disorders.

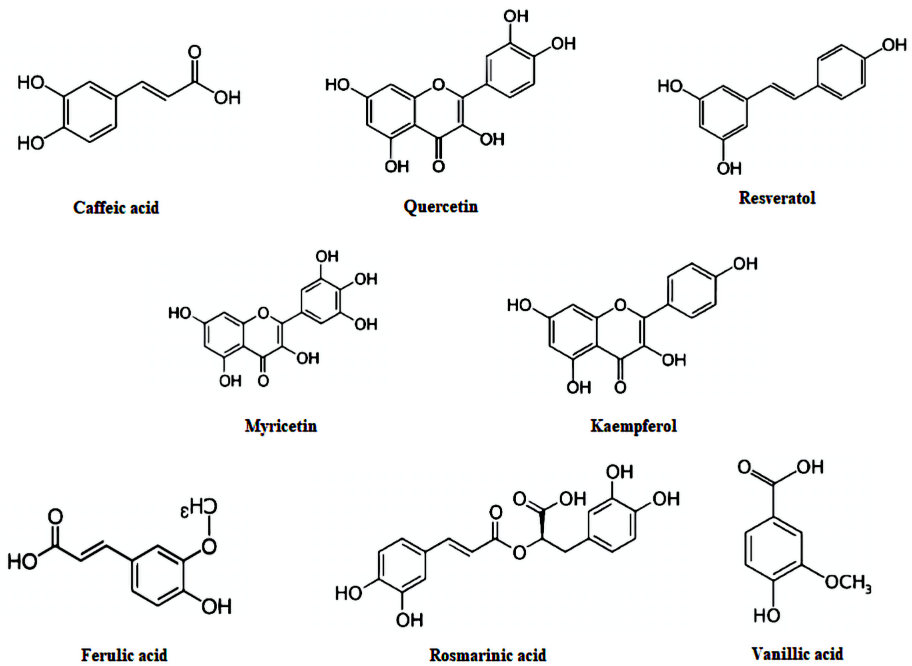

Fig 1: Chemical structures of some of the polyphenols found in Saperavi shoots

2. MATERIALS AND METHODS

2.1. *Preparation of extract*

Saperavi grapevine shoots were collected in Alvani village, Georgia, in June 2021. The plant material was well-dried and grinded. Preliminary studies demonstrated that 50% ethyl alcohol is the best solvent for polyphenol extraction. During this experiment, we used a cold extraction process. Accordingly, 50g of plant material was placed in a glass flask, and 500 ml of 50% alcohol was added. Flask was shaken on a laboratory shaker for 2 hours, following 1 hour of rest and two additional hours of shake. The extract was left to rest for the next 12 hours and filtered. Afterward, alcohol was evaporated, and total polyphenol content was measured using Folin-Ciocalteu reagent utilizing Gallic acid on Spectrophotometer i9 Hanon instruments (wavelength 765 nm)[23]. The prepared extract was given to experimental group III of animals orally instead of water.

2.2. *Determination of the Viscosity of the extract*

50 ml of prepared extract was placed in the beaker, and rheological properties were measured using Rotary Viscometer LVDV-1T according to instructions. To obtain the result, Spindler #3 with 100.0 rpm was used. The Viscosity of Saperavi shoots extracted at 20.8°C was 89 cP.

2.3. *Experiment*

Healthy adult Wistar rats weighing 180-200g were used for the study. The animals were obtained from the "Bio-Medical Research Supporting Association," Tbilisi, Georgia. Food and water were given *ad libitum*. The room with animal cages was well-ventilated with a 12 h light-dark cycle and 25°C temperature throughout the study period. Rats were given seven days to adapt to a new environment condition before the initiation of the experiment. The research was carried out with the permission of the Animal Research Ethics Committee of Tbilisi State Medical University and in full compliance with its regulations (order from January 2019, reg No: 07/03). A total of 30 rats was divided into three groups: Group I: Control group – animals received regular chow diet (n=6); Group II: Animals received hypercholesterolemic diet (HCD) (n=12); Group III: Animals received HCD together with Saperavi shoots extract (SSE; n=12). Regular chow diet and HCD (diet enriched with 1% cholesterol) were obtained from "Synergy-Bio," Nanjing, Jiangsu Province, China. Experiments were started by weighing animals in all groups. Next 30 days, the control group was fed an ordinary chow diet, while the experimental groups received HCD. Groups III received SSE instead of water *ad libitum*. On the 31st day, fasted overnight, animals were euthanized (sodium Pentobarbital, 60mg/kg, i.p), and blood samples were collected from the superior vena cava to investigate biochemical parameters. Total cholesterol levels, as well as HDL-C, LDL-C, and TG, were evaluated using CLINDIAG SA-20 Semi-Automatic Biochemistry Analyzer (Belgium) as per instruction of the manufacturer using Biolabo test kits (France). Liver function was assessed with alanine transaminase (ALT) and aspartate transaminase (AST) in serum samples using the Biolabo colorimetric assay kits. Results are summarized in table 1.

Table I: Body weight and biochemical parameters in rats on the 31st day of the experiment

	Group I	Group II	Group III
Body weight (g)	224.6±5.08	253.15±5.15*	244.4±3.43**
T. CHOL (mmol/L)	1.88±0.47	4.02±0.13*	3.8±0.15**
HDL-C (mmol/L)	0.54±0.1	0.9±0.17*	1.19±0.14**
LDL-C (mmol/L)	1.16±0.39	2.06±0.07*	1.73±0.37**
TG (mmol/L)	0.49±0.12	2.12±0.23*	1.91±0.4**
ALT (IU/L)	27.83±4.83	30.33±4.67*	28.16±3.25**
AST (IU/L)	99.83±5.77	129.5±4.76*	101.83±2.85**

Difference between I and II groups.
***** Difference between II and III groups.***

Table I demonstrates body weight, lipid profile, ALT, and AST of researching and control groups on the 31st day of study. The obtained results confirmed the development of hypercholesterolemia in experimental groups and demonstrated the protective effect of SSE in group III. Serum lipid profile on the 31st day of the experiment demonstrated the efficiency of the selected diet as hypercholesterolemia developed in both experimental groups (normal range is 0.5 – 3.0 mmol/L[24]). At the same time, a positive protective effect of polyphenol-rich SSE was established, as group III has developed a lower value of hypercholesterolemia than group II. Herewith, the level of HDL-C should be highlighted, as in group III receiving SSE instead of water, a higher level was kept compared to group II. In the final ten days of the experiment, all groups were fed with a regular chow diet but received different treatment per group: Group I – no treatment, group II received 10mg/kg Atorvastatin suspension[25] once a day p.o, group III proceeded its treatment with SSE. Data on body weight and biochemical parameters are presented in table 2.

Table II: Body weight and biochemical parameters in rats on the 41st day of the experiment

	Group I	Group II	Group III
Body weight (g)	244.76±4.18	279.75±5.01*	268.42±4.11**
T. CHOL (mmol/L)	1.93±0.31	2.69±0.19*	2.86±0.1**
HDL-C (mmol/L)	0.7±0.13	0.85±0.14*	1.17±0.16**
LDL-C (mmol/L)	1.02±0.37	1.36±0.23*	1.36±0.38
TG (mmol/L)	0.45±0.18	1.02±0.21*	0.72±0.57**
ALT (IU/L)	27.33±3.88	33.83±3.31*	28.33±2.35**
AST (IU/L)	101.5±4.32	133.16±4.07*	105.17±3.48**

Each value is expressed in mean ± SD; (n=6).
****Difference between I and II groups.***
***** Difference between II and III groups.***

Table II demonstrates body weight, lipid profile, ALT, and AST of researching and control groups on the 41st day of study. Both groups demonstrated reduced cholesterol levels to the normal range; however, results in group III are significantly better than those in group II. Group III demonstrated a smoother reduction of serum cholesterol level than group II, which received Atorvastatin; nevertheless, it is noteworthy that both groups demonstrated a reduction of cholesterol level to the normal range. Additionally, HDL-C high level was maintained in group III, which received treatment with SSE for a total of 40 days.

2.4. Statistical Analysis

Received results are expressed as the mean ± SD. Differences between groups were analyzed using a one-tailed Student's *t-test*, and values were considered statistically significant at a $P<0.05$.

3. RESULTS AND DISCUSSION

Numerous polyphenols have been reported to have hypocholesterolemic effects[26-28]. Saperavi grapevine shoots extract has been confirmed to be rich in different polyphenols. Therefore, the current study was initiated to investigate the lipid-lowering effect of Saperavi shoots extract (SSE) compared with a daily dose of Atorvastatin, a recognized hypolipidemic agent. The first step of the experiment was the initiation of hyperlipidemia in rats. During 30 days' animals received HCD, and group III additionally received SSE instead of water, *ad libitum*. The lipid profile was analyzed on the 31st day, and the results confirmed the development of dyslipidemia in groups II and III. However, lower values of T.Chol, LDL-C, and TG, along with the higher value of HDL-C, were observed in group III, which should be linked to the expressive hypolipidemic activity of SSE polyphenols. The total body weight of animals in group II was higher than in those of group III due to the established anti-dyslipidemia effect of polyphenols. These results are consistent with earlier published studies regarding the anti-dyslipidemia effects of polyphenols of different origins[29-31], where experimental animals showed an increase in body weight and development of dyslipidemia similar to in the current study. Namely, in the research "hypocholesterolemic effects of phenolic extracts and purified hydroxytyrosol recovered from olive mill wastewater in rats fed a cholesterol-rich diet," authors outlined receipt of lower concentrations of total cholesterol and LDL-C in animals supplemented with polyphenols[29]; Similarly, In the study "Effect of polyphenol, flavonoid, and saponin fractions from Thymus atlanticus on acute and chronic hyperlipidemia in mice" it was concluded, that "administration of the fractions from T. atlanticus, which are rich in phenolic compounds, particularly rosmarinic acid, may have a beneficial role in hyperlipidemia through decreasing proatherogenic lipids level and increasing anti-atherogenic HDL-C"[30]. In the current study, along with relatively low values of total cholesterol (5.47%), LDL (16%), and TG (9.9%), better results for HDL value were observed for group III. HDL-C was 25% higher than in animals from group II, which can be considered a significant benefit of SSE administration. A correlative increase of HDL-C level was observed in several studies conducted to evaluate different phenol actions on lipid profile[33-35], where authors relied on the current approach that increases in HDL-C are as beneficial in treatment as a decrease of non-HDL-C. Authors in the study "Olive Oil Polyphenols Enhance High-Density Lipoprotein Function in Humans" demonstrated that "Olive oil polyphenols promote the main HDL anti-atherogenic function, its cholesterol efflux capacity"[33]; In the study of Zeni A.L et al., "Morus nigra leaves showed capability to diminish TC, TG, VLDL, and LDL with augmentation of HDL levels in hyperlipidemic rats"[34]. The next milestone of the current study was regulating lipid profile to revert to the normal range and evaluate the possibility of using SSE as a treating agent. Therefore, animals were switched to a regular chow diet and treated appropriately. Group I proceeded with no treatment for serving as a control again; Group II received 10mg/kg/day Atorvastatin suspension *p.o.*; Group III proceeded with its treatment with SSE. After ten days of medication, blood samples were collected and analyzed. Group III demonstrated a smoother reduction of serum cholesterol level than group II, which received Atorvastatin; nevertheless, it is noteworthy that both groups demonstrated reduced cholesterol levels to the normal range. Additionally, HDL-C high level was maintained in group III, which received treatment with SSE for a total of 40 days. The transformation of the lipid profile in groups II and III is presented in figure 2.

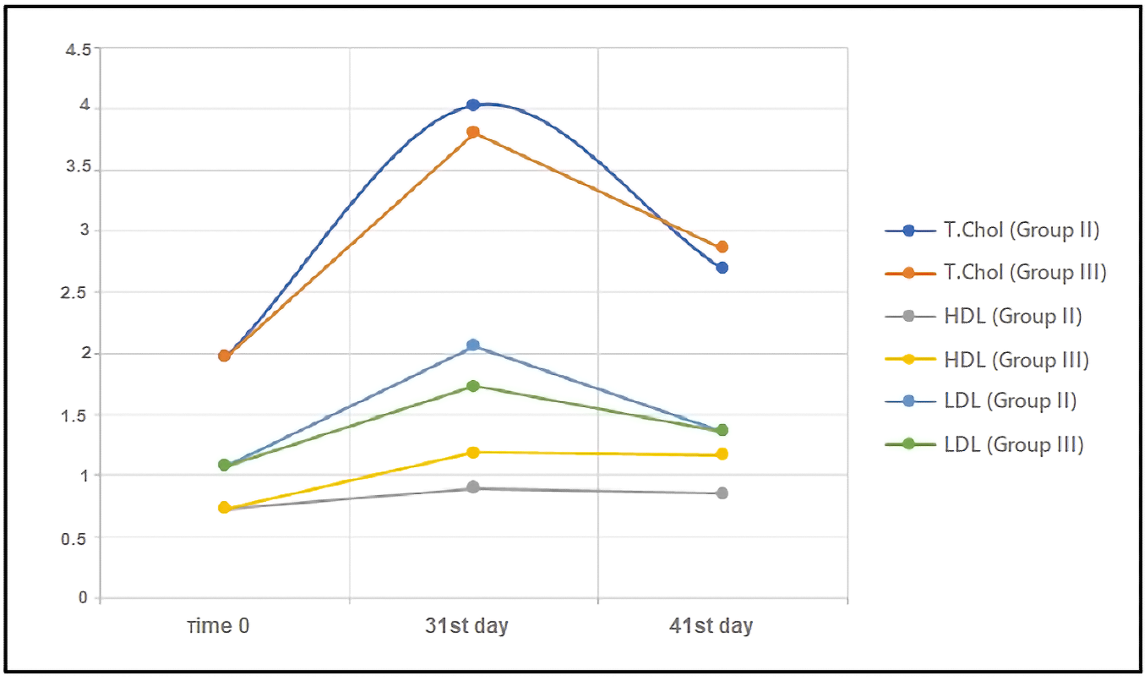

Fig 2: Comparison of lipid profile transformation in Group II and Group III

These findings agree with the latest results regarding the potential implication of natural polyphenols in dyslipidemia treatment strategies[36-38]. Furthermore, achieved findings form a solid base for further research of SSE, as it was done for numerous natural extracts containing different polyphenols[39,40].

4. CONCLUSION

The present study analyzed the possibility of superior utilization of wasted grapevine shoots in contrast with the current approach. In addition, observed lipid profile regulation levels in *In Vivo* research demonstrated that phytomedicines could have a significant role in treatment along with established medicines like Statins. In the experiment, Saperavi shoot extracts positively reduced TC, LDL, and TG, along with increased HDL content in HCD-fed rats. Notably, the end of the experimental group of animals receiving SSE demonstrated a smoother reduction of cholesterol levels than the group receiving Atorvastatin. Still, in all groups, normal cholesterol level was achieved.

In conclusion, these findings support the potential of currently wasted grapevine shoots as a source of biologically active ingredients. Based on the study results, Saperavi shoot extract can be considered a cost-effective candidate for phytomedicine supplementing and treating cardiovascular diseases associated with lipid profile disorders. Furthermore, obtained results proved the importance of further deep research on SSE and its potential benefits for dyslipidemia prevention and treatment.

5. FUNDING ACKNOWLEDGEMENT

This research No PHDF-21-1607 has been supported by Shota Rustaveli National Science Foundation (SRNSFG).

6. AUTHORS CONTRIBUTION STATEMENT

N.B., T.M., and N.M. devised the project and the main conceptual ideas. K.S. and M.T. performed manipulations on plant sources to receive and standardize SSE. N.B. and N.M. performed experiments on animals. T.M. and N.M. were supervising the findings of the work. All authors discussed the results and contributed to the final manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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