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Original Article

Antioxidant and Anti-hypercholesterolemic potential of *Vitis vinifera* leaves

Sushma Devi and Randhir Singh*

ABSTRACT

Background: Grapes (*Vitis vinifera*) are universally appreciated fruit for their delicacy, nutrition and accepted as functional food. The objective of the present study is to evaluate antioxidant and anti-hypercholesterolemic potential of *Vitis vinifera* leaves extract. **Methods:** Qualitative and quantitative phytochemical screening of methanolic (VVME) and aqueous (VVAE) extract was carried out to identify the phytoconstituents. Antioxidant potential was evaluated by employing *in-vitro* and *in vivo* assays. The anti-hypercholesterolemic activity was evaluated by inducing hypercholesterolemia with high cholesterol diet for 21 days in experimental animals. **Results:** In VVME, total tannins, total flavonoids and total phenolic contents were found to be present in major amount. Both extract has significant *in-vitro* and *in-vivo* antioxidant efficacy. Different doses i.e. 100, 200 and 400 mg/kg of VVME and VVAE significantly attenuated the lipid levels. Moreover, VVME was found to be more effective as compared to VVAE and also, effectiveness was confirmed with histological results. **Conclusion:** It can be concluded that antioxidant and anti-hypercholesterolemic efficacy of *Vitis vinifera* might be due to presence of antioxidant property and active phytoconstituents.

Key words: *Vitis vinifera*, Antioxidant, Hypercholesterolemia, Cholesterol Diet, Total Phenolic Content Etc.

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INTRODUCTION

Nowadays, functional food is an emerging field in food science. Functional foods are known to have positive effects on human health, such as prevention of CVD, cancer, reducing cholesterol risk and regulating the digestive system. Grape seed, fruit and their extract are already used as functional food.[1] Moreover, research and commercial activities on functional food and/or ingredients are in great demand. However, there are few reports on the antioxidant activities and antidyslipidemic effects of *Vitis vinifera*, although it is well known that many plants have antioxidant and free radical scavenging activities.

Free radical oxidative stress, usually resulting from deficient natural anti-oxidant defenses, has been implicated in the pathogenesis of a wide variety of clinical disorders, such as the degenerative diseases, aging and the progressive decline in the immune functions. The pathological roles of free radicals have been implicated in a wide range of inflammatory diseases.[2] As well as, it has been reported that hypercholesterolemia is increased free radical production and reduced free radical scavenging effect. Therefore, certain natural products with antioxidant activities may have potential anti-hypercholesterolemia actions. So, the present work was designed to evaluate the antioxidant and anti-hypercholesterolemic potential of VVME and VVAE of *Vitis vinifera* leaves.

MATERIAL AND METHODS

Chemical used

Cholesterol (Hi Media) cholic acid (Hi Media), Simvastatin (sample from beta drugs pvt. ltd), Aluminium chloride (Nice chemicals), Ascorbic acid (Sigma), DPPH (SD Fine), Folin ciocalteu reagent (Sigma), Gallic acid, Methanol (Nice chemicals) and spectrophotmetric analysis was carried out by using UV spectrophotometer (Shimadzu). Erba diagnostic kits were used for estimation of total cholesterol, total triglyceride and HDL levels in serum.

Plant material

Fresh leaves of *Vitis vinifera* was collected on April 2013 from the Tau Devilal National herbal park, Khizrabad, Haryana, India and authenticated by Dr. Shiddamallayya N., National Ayurveda Dietetics Research Institute, Banglore, India (specimen number RRCBI-MUS-125).

Preparation of extract

Leaves of *Vitis vinifera* was washed in water and shade dried. The dried leaves were grinded into coarse powder. Then, plant material was packed into soxhlet and extraction was carried with soxhlation for 72 hrs using methanol aqueous. The extract was concentrated using vacuum rotary evaporator at 40°C, dried and stored in a refrigerator at 4°C

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throughout the duration of study.[3] The % yield of VVME and VVAE was found as 8.4% and 11.2% w/w, respectively.

Qualitative estimation of Phytoconstituents

The qualitative phytochemical screening of VVME and VVAE was carried out to determine phytoconstituents present by using standard test.[4-5]

Quantitative estimation of Phytoconstituents

Total phenolic content [6]

Total phenolic content in the extracts was determined with Folin ciocalteau reagent using gallic acid as a standard. Different concentrations (50, 100, 150, 250, and 500 mg/l) of gallic acid solutions were prepared. 1 ml of solution was taken in 25 ml volumetric flask, 10 ml distilled water was added to each and then 1.5 ml of the folin ciocalteu reagent was added and mixed well. After 8-10 min, 4 ml sodium carbonate solution (7.5% w/v) was added and volume was adjusted upto 25 ml. Solutions was kept at 40°C for 30 min and absorbance was determined at 765 nm against the blank and plot absorbance vs concentration. The concentration of total phenols was expressed as mg/g of gallic acid equivalent dry weight and experiment was performed in triplicate.

Total flavonoids content [7]

The aluminum chloride colorimetric method was used to determine the flavonoid content of plant extracts. 0.5 mg/ml of extract solution was added into 1.5 ml of methanol. 0.1 ml of 10% aluminium chloride was added followed by incubation for 5 minutes after which 0.1 ml potassium acetate (1 M). Finally, 2.8 ml distill water was added and shaked and kept at room temperature for 30 min. Absorbance of the sample was noted at 420 nm with UV spectrophotometer. Rutin was used as the standard for the calibration curve. From the rutin stock solution 10, 20, 30, 50 and 100 mg/l solutions was prepared. Similarly, in place of extract sample, 0.5 ml of rutin solution was added. Flavonoid contents were expressed as mg/g rutin equivalent dry weight. A yellow color indicated the presence of flavonoids. From the standard graph, the amount of total flavonoids content in the sample as per absorbance values was calculated and expressed as rutin equivalents (mg/g).

Total tannin content [8]

Total tannin content was estimated using vanillin hydrochloride method. Vanillin hydrochloride (mix equal volume of 8% HCl in methanol and 4% vanillin in methanol) was freshly prepared. 1 ml of extract solution was added in 5 ml vanillin hydrochloride reagent and allowed to stand for 20 min. Rutin stock containing 1 mg rutin/ml of different concentrations 10, 20, 30, 50 and 100 mg/ml was prepared with methanol and absorbance was measured at 500 nm. Total tannin content in the sample was calculated from the standard graph.

Total alkaloids content [9]

1 mg extract was dissolved in dimethyl sulphoxide (DMSO) and 1 ml of 2 N HCl was added and filtered. Transferred the sample into separating funnel and 5 ml of bromocresol green solution was added and 5 ml of phosphate buffer (pH 4.7). 1, 2, 3 and 4 ml chloroform was added by vigorous shaking, collected in volumetric flask (10 ml) and volume was made up with chloroform. Stock solution of atropine was prepared and concentrations 20, 40, 60, 80 and 100 µg/ml was prepared in the similar manner as extract. Absorbance was measured at 470 nm with an UV/Visible spectrophotometer and expressed as mg of atropine/g of extract.

Total saponins content [10]

1 ml of plant extract sample was added in methanol (80%) and 2 ml of vanillin in ethanol and mixed well. Then, 2 ml H_2SO_4 (70%) was added and heated at temperature 60°C in water bath for 10 min. Absorbance of sample was noted at 544 nm against blank. Diosgenin was used as standard and calibration curve was prepared. The different concentration of Diosgenin 10, 20, 40, 80, 150 µg/ml was used for preparing standard curve. From the standard graph, the amount of saponins in the sample as per absorbance values was calculated and expressed as diosgenin equivalents (mg/g).

Total steroids content [10]

1 ml of plant extract sample was added in a 10 ml volumetric flask and 2 ml H_2SO_4 (4 N) and 2 ml ferric chloride (0.5% W/V) was added into the extract. Then, 0.5 ml potassium hexacyanoferrate (III) solution (0.5%) was added. The mixture was heated in the water bath at temperature 70±20°C for 30 min. The volume was made up with distill water and absorbance was noted at 780 nm against blank. Cycloartenol was used as standard and different concentration of cycloartenol (10, 20, 40, 80, 160 µg/ml) was used for absorbance and standard curve. From the standard graph, the amount of steroid in the sample as per absorbance values was calculated and expressed as cycloartenol equivalents (mg/g).

Total terpenoids content [11]

Take 100 g of plant powdered material was soaked in alcohol for 20-25 h. Then, filtered and extracted with petroleum ether. Extract was evaporated, weighted and ether extract treated as total terpenoids content.

Antioxidant activity (*in vitro*)

DPPH radical scavenging activity [12]

The hydrogen-donating ability of each extract was examined according to the method previously described in the presence of a DPPH stable radical. Ascorbic acid at various concentrations (10-200 µg/ml) was used as standard. The antioxidant activity was calculated as % inhibition using formula:

$\%\ \text{inhibition} = (A_{blank} - A_{sample})/A_{blank} * 100$

A_{blank} = absorbance of the control

A_{sample} = absorbance in the presence of the extract.

Superoxide radical scavenging activity [13]

NBT (Nitro blue tetrazolium reagent) was used to generate the superoxide radical by auto oxidation of hydroxylamine hydrochloride and reduced into nitrite. Different concentrations (20-500 µg/ml) of sample were prepared and 1 ml of 50 mM sodium carbonate, 0.4 ml of 24 mM NBT 0.2 ml of 0.1 mM EDTA solution was added. The absorbance was recorded at 560 nm in 0 min. The reaction starts after adding 0.4 ml of 1 mM hydroxylamine hydrochloride in the above solution and incubated at 25°C for 15 minute.

Hydroxyl radical scavenging activity [14]

Hydroxyl radical generation by phenylhydrazine was measured by 2-deoxyribose degradation assay. 1 mM deoxyribose, was added in 50 mM phosphte buffer (pH 7.4) containing 0.2 mM phenylhydrazine hydrochloride in a test tube. Incubation was terminated after 1 hour. 1 ml of TCA (2.8%) and thiobarbituric acid (1% w/v) was added in reaction mixture and mixture was heated for 10-15 min on water bath, cooled and the absorbance was measured at 532 nm.

Nitric oxide scavenging activity [15]

For estimation of nitric oxide scavenging activity of extracts, nitrite detection method was used. Sodium nitroprusside (10 mm) in 0.5 m phosphate buffer (pH 7.4) used as source of NO in an aqueous solution. Further, the sample was incubated for 60 min at 37°C and Griess reagent

(a-napthyl-ethylenediamine 0.1% in water and sulphanilic acid 1% in H_3PO_4 5%) was added. For the control reagent, same reaction mixture prepared without the extract but equivalent amount of distilled water.

Antioxidant activity index [16]

Antioxidant activity index (AAI) was determined by the 2,2-diphenyl-1-picrylhydrazyl method proposed by Scherer and Godoy. Antioxidant activity index (AAI) was calculated by the formula:

AAI = final concentration of DPPH in control /IC_{50}

According to the AAI scale:

AAI < 0.5 poor antioxidant activity

0.5 < AAI < 1.0 moderate antioxidant activity

1.0 < AAI < 2.0 strong antioxidant activity

AAI > 2.0 very strong antioxidant activity

Animals

Wistar rats (both sex) were used in the study and experimental protocol was duly approved by Institutional Animal Ethics Committee (MMCP/IAEC/13/36). Animals were kept as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA) in Department of Pharmaceutical Sciences, Maharishi Markandeshwar University, Ambala, India. Animals were fed normal chow diet and *ad libitum* under controlled environmental condition of temperature (24-28°C), relative humidity 60-70% and natural light/dark cycle (12:12).

Antioxidant activity (*in vivo*) [17]

Both extracts were administered in experimental animals for 7 days with different doses (100, 200 and 400 mg/kg) and serum was separated with centrifugation at a speed of 3000 rpm for 10 min. The level of reduced glutathione and catalase was measured in serum.

Estimation of reduced glutathione [18]

Reduced glutathione level was estimated by Moran *et al.* modified method. 1 ml of serum was added in 6 ml phosphate buffer 0.2 M (pH 8.0) and 1 ml DTNB 0.6 mM. Then, mixture was incubated at room temperature for 10 min. The absorbance was noted at 412 nm and standard curve was prepared by using different concentrations (0-50 µg/ml) of GSH. GSH concentration was calculated using the dilution factor and expressed as µg/mg of protein.

Estimation of catalase activity [19]

The reaction mixture (2 ml) was containing 1.95 ml H_2O_2 (10 mM) in 50 mM phosphate buffer (pH 7.0). 0.05 ml supernatant was added and reaction started. The absorbance was noted at 240 nm and phosphate buffer (50 mM, pH 7.0) was used as standard. The extinction coefficient of 0.04 $mM^{-1}cm^{-1}$ was used and data was expressed as U/mg protein. The unit of catalase is defined as the quantity, which decomposes 1.0 µmole of H_2O_2 per min at pH 7.0 at 25°C, while this H_2O_2 concentration falls from 10.3 to 9.2 mM.

Induction of hypercholesterolemia with cholesterol diet [20-21]

Wistar Albino rats (200-220 g) were procured under controlled environmental conditions. Atherosclerosis was induced by administration of cholesterol diet (cholesterol 2% w/w and cholic acid 0.5% w/w along with basal diet) for 21 days. Simvastatin (dose 10 mg/kg), VVME and VVAE (dose 100 mg/kg, 200 mg/kg and 400 mg/kg) were administered for 21 days.

Changes in body weight

The change in the body weights was recorded weekly and % change in body weights was calculated using formula:

% change in weight = (final weight- initial weight)/initial weight * 100

Biochemical estimations

At the end of study, blood was collected via retro-orbital plexus and centrifuged at 3000 rpm for 10 min and serum was separated. The serum glucose, triglycerides, total cholesterol and HDL level was measured using enzymatic kits. The levels of LDL and VLDL were calculated using Friedewald equation.

Liver functions test [22]

The levels of SGOT and SGPT were also analysed in the blood samples at the end of study using diagnostic kits.

Histopathological studies

Then, animals were sacrificed and heart was isolated for histopathology. A portion of heart tissue was dissected out and fixed in 10% formalin solution and histopathological studies were carried out.

Atherogenic Index [23]

Atherogenic index and % protection was also calculated at the end of study using formulas:

$$\text{artherogenic index} = \log\left(\frac{\text{total triglyceride}}{\text{total HDL cholesterol}}\right)$$

$$\text{Protection (\%)} = \frac{\text{atherogenic index of control} - \text{atherogenic index of treated}}{\text{atherogenic index of control}} \times 100$$

Statistical analysis

All the data were shown mean values and represented as mean±SEM. Statistical analysis was done with Dunnett's multiple comparison tests using Graph pad Instat Demo software (version 3.10). In statistical analysis, $p<0.05$ was considered to be significant; b = *vs* cholesterol control; c = *vs* 100 mg/kg dose; d = *vs* 200 mg/kg dose; p< 0.05 = *; p< 0.01= ^; p< 0.001= #.

RESULTS

Qualitative and Quantitative estimation of phytoconstituents

VVME and VVAE revealed that flavonoids, phenolic, tannins, saponins, steroids and terpenoids were present. Moreover, alkaloids were present in VVAE only (Table 1). In VVME, total tannins content, total flavonoids content and total phenolic content was found to be present in major amount (Table 2).

In vitro antioxidant activity of VVME and VVAE

In DPPH scavenging assay, 500 µg/ml of VVME and VVAE produced 68.25% and 62.08% inhibition. Whereas, in hydroxyl radical scavenging activity, 500 µg/ml of VVME and VVAE produced 54.06% and 52.75% inhibition, respectively. In superoxide radical scavenging assay ascorbic acid, VVME and VVAE produced 76.15%, 60.50% and 54.92% inhibition, respectively. In nitric oxide radical assay, ascorbic acid, VVME and VVAE have 86.20%, 65.07% and 56.09% inhibition, respectively. Antioxidant effect of VVME in different assay is in following order:

DPPH > Nitric oxide > Superoxide > Hydroxyl radicals. VVME was found to have strong antioxidant effect than VVAE. The antioxidant effect was found in following order: ascorbic acid > VVME > VVAE.

DPPH generate free radical and widely used to determine the antioxidant potential of various drugs and plant extracts. The inhibition mechanism of lipid oxidation is one of the known free radical scavenging activity that occurs exogenously in human body.[24,25] Although superoxide anion is a weak oxidant and has important role in the generation of other ROS, like hydroxyl radical, hydrogen peroxide, or singlet oxygen which contribute to oxidative stress in living systems. [26] The present study suggested that there is a strong correlation between superoxide and hydroxyl radical scavenging activity and phenolics (22.27±1.69), flavonoid (34.10±0.26) and tannins (33.27±0.32) contents of VVME. Nitric oxide (NO) scavengers from the extracts compete with oxygen, leading to reduced production of nitrite ions. There is no significant correlation was found between NO radicals scavenging activity and phenolics. Hence, bioactive substances other than phenolics, tannins and flavonoids may be the reason for scavenging activity in extracts.

IC_{50} values of ascorbic acid, VVME and VVAE is shown in table 3. IC_{50} is used to express the concentration or amount of samples/extracts desired to scavenge 50% of the free radicals. The scavenging activity of a sample/extract is inversely proportional to the IC_{50} value.

Antioxidant Activity Index (AAI) of ascorbic acid, VVME and VVAE was found to be 4.43>2.05>1.48 respectively. According to AAI, both ascorbic acid and VVME lies into very strong category and VVAE lies into strong antioxidant category. [16]

In vivo antioxidant activity

Administration of different doses of VVME and VVAE significantly elevated the serum catalase level and serum reduced glutathione level. The increase in the amount of serum catalase was found to be dose dependent. In VVME 400 mg/kg, the level of reduced glutathione and catalase was found maximum (6.32±1.12 µg/mg of protein and 7.55±2.66 µM/min/mg of protein) as compared to other groups (Table 4). As a preliminary step towards unveiling the mechanism of actions of these extracts in oxidative stress, their effects on common oxidative stress marker enzymes such as serum glutathione and catalase was estimated. The significant increase in serum GSH suggested that the activation of the GSH synthetic pathway does not occur as outcome of an increased production of free radicals and with non-significant depletion of the total protein. [2] Also, it could be indirect pathway that one or more constituents of extract probably have some biochemical action on GSH production or affect the reduction process of GSSG to GSH. In addition, molecular evidence also suggest that the ability of some phenolic compounds to activate c-glutamylcysteine synthetase (a rate-limiting enzyme in GSH synthesis).[27] According to a study, due to plant bioactive secondary metabolites, the increment in GSH concentration contributes to the chemoprevention against environmental carcinogens.[28] Glutathione is vital intracellular free radicals scavenging agent and co-substrate for various enzymes. Also, it has an important role in the degradation of H_2O_2 and molecule itself undergoes oxidation process from its reduced state GSH to its oxidized state GSSG. It is active against free radicals, peroxides and other toxic compounds and protects the cells. GSH majorly involve into metabolism, catalysis and transportation. In kidney, GSH involve in the reabsorption of amino acids during transportation.[29] The enzymatic antioxidant systems such as catalase, glutathione reductase, play a coordinated role in the prevention of oxidative damage by ROS. On the other hand, catalases have heme proteins and protect the cells from toxic effects of ROS. They convert H_2O_2 into water and molecular oxygen. During aerobic metabolism, superoxide anion is being produced as a byproduct. Superoxide dismutase breaks it up into H_2O and H_2O_2 and then H_2O_2 is converted to H_2O and O_2 by catalase.[30] Administration of extracts enhanced the antioxidant enzymes (GSH and catalase) activity in a dose dependent manner. The improved antioxidant enzymes activity may offer an effective defense system and prevent from the damage of free radicals [2]

Finally it can be concluded that secondary metabolites act as small molecular weight antioxidants and perform directly as antiradical agent or break chain reaction of free radical and interact with transition metals. Plants secondary metabolites can act indirectly include inhibition of ROS-generating enzymes such as xanthine oxidase or induce nitric oxide synthase or up-regulate the SOD or other enzymes activity.[31] Phenolic compounds have the capability to adsorb or neutralize or quench ROS. Also, flavonoids or related compounds exhibit *in vitro* and *in vivo* antioxidant potential.[32]

Change in body weight of experimental animals

Hyperlipidemia or hypercholesterolemia in rats can be induced by supplementing cholesterol diet (sub-acute model).[33] Excessive cholesterol feeding leads to susceptibility to hypercholesterolemia and arteriosclerosis and further promotes the development of obesity and dyslipidemia in both humans and rodents by altering the plasma cholesterol and triglyceride levels.[34] According to literature, hypercholesterolemia animals are used to study the cholesterol homeostasis as convenient models. As well as, to understand the association between cholesterol metabolism disorders, a therogenesis or possible treatments to reduce lipid levels in drug trials.[35] Also, administration of cholesterol diet significantly changes in the body weight of rats during the experimentation. The body weights of animals were measured weekly during 21 days of treatment. The % change in body weight of VVME 100 mg/kg, 200 mg/kg and 400 mg/kg group animals were found to be 14.6%, 12.9% and 13.2%, respectively. The % change in body weight of VVAE 100 mg/kg, 200 mg/kg and 400 mg/kg groups were found to be 23.8%, 23.7% and 19.8% respectively.

Effect of VVME and VVAE on glucose level

200 mg/kg and 400 mg/kg of VVME statistically lowered the glucose level as compared to cholesterol control. (Figure 1)

Effect of VVME and VVAE on lipids level

The total cholesterol level was found to be significantly elevated in experimental animals and different doses of VVME and VVAE produced a significant attenuation in serum cholesterol level. The maximum attenuation in total cholesterol level was found in VVAE 200 mg/kg (140.2 mg/dl). The total triglyceride level was elevated in the experimental animals and administration of different doses of VVME and VVAE significantly attenuated the triglyceride level. The maximum attenuation in triglyceride level was found in VVME 400 mg/kg group (115.5 mg/dl). Similarly, LDL level was also found to be significantly elevated in experimental animals and different doses of VVME and VVAE produced a significant attenuation in LDL level. The VLDL level was found to be significantly attenuated in cholesterol control as compared to normal control. Whereas, HDL level was found to be decreased in experimental animals and administration of different doses of VVME and VVAE significantly elevated the HDL level after 21 days treatment. (Figure 2-6)

High cholesterol diets acts as extrinsic inducer and significantly increase the cholesterol, triglyceride, LDL levels and decrease HDL level. Increase in LDL has been indicated one of the risk factors in development of atherosclerosis and other related cardiovascular disorders. [36] High triglyceride levels also a marker and important risk factor that influences lipid deposition and clotting mechanisms. Numerous experimental

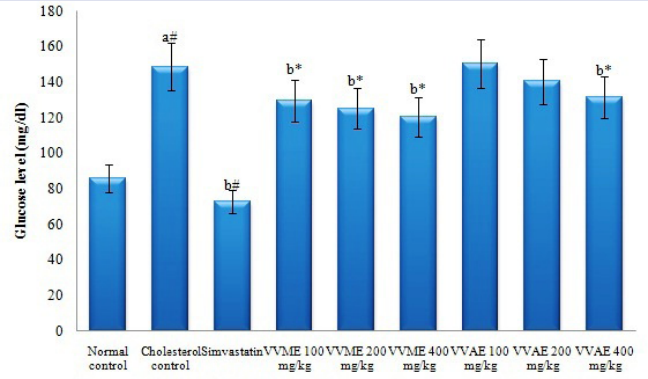

Figure 1: Effect of VVME and VVAE on glucose level in cholesterol induced hypercholesterolemia. Values are represented as mean±SEM, n=6. In statistical analysis, p<0.05 was considered to be significant; a = vs normal control; b = vs cholesterol control; p< 0.05 = *; p< 0.01= ^; p< 0.001= #

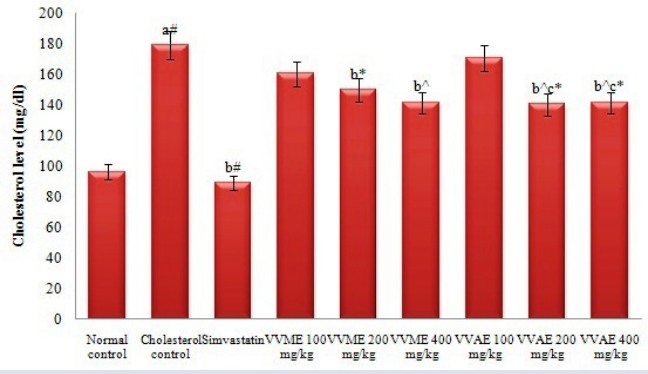

Figure 2: Effect of VVME and VVAE on cholesterol level in cholesterol induced hypercholesterolemia. Values are represented as mean±SEM, n=6. In statistical analysis, p<0.05 was considered to be significant; a = vs normal control; b = vs cholesterol control; p< 0.05 = *; p< 0.01= ^; p< 0.001= #.

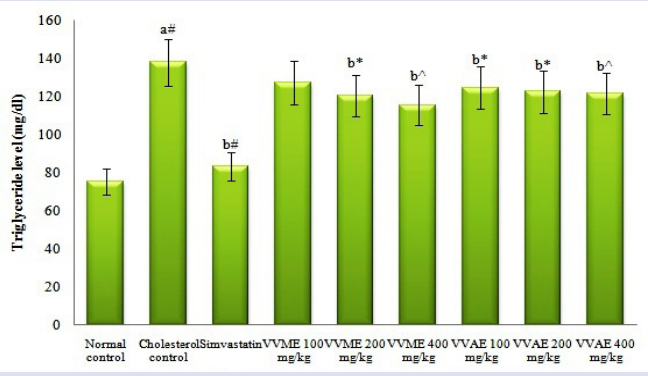

Figure 3: Effect of VVME and VVAE on triglyceride level in cholesterol induced hypercholesterolemia. Values are represented as mean±SEM, n=6. In statistical analysis, p<0.05 was considered to be significant; a = vs normal control; b = vs cholesterol control; p< 0.05 = *; p< 0.01= ^; p< 0.001= #

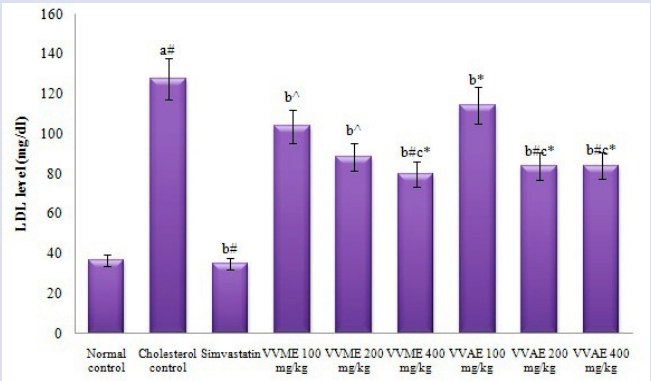

Figure 4: Effect of VVME and VVAE on LDL level in cholesterol induced hypercholesterolemia. Values are represented as mean±SEM, n=6. In statistical analysis, p<0.05 was considered to be significant; a = vs normal control; b = vs cholesterol control; c = vs 100 mg/kg dose; p< 0.05 = *; p< 0.01= ^; p< 0.001= #.

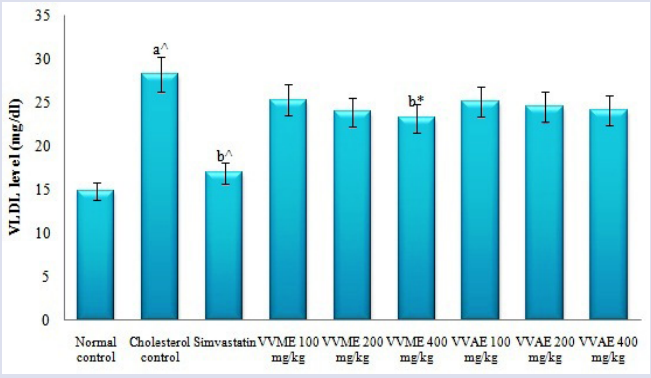

Figure 5: Effect of VVME and VVAE on VLDL level in cholesterol induced hypercholesterolemia. Values are represented as mean±SEM, n=6. In statistical analysis, p<0.05 was considered to be significant; a = vs normal control; b = vs cholesterol control; p< 0.05 = *; p< 0.01= ^; p< 0.001= #.

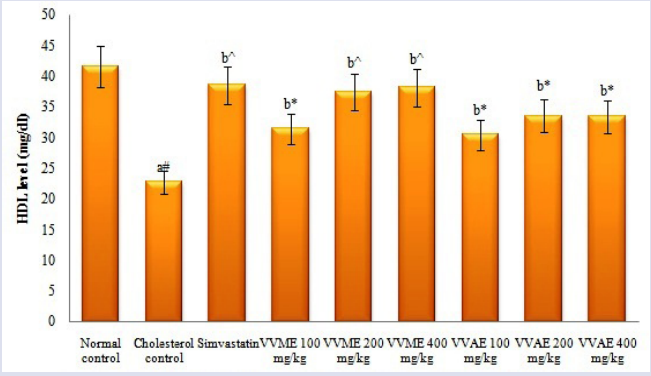

Figure 6: Effect of VVME and VVAE on HDL level in cholesterol induced hypercholesterolemia. Values are represented as mean±SEM, n=6. In statistical analysis, p<0.05 was considered to be significant; a = vs normal control; b = vs cholesterol control; p< 0.05 = *; p< 0.01= ^; p< 0.001= #.

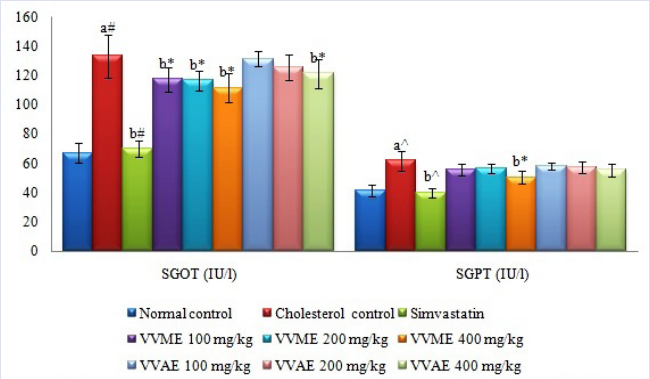

Figure 7: Effect of VVME and VVAE on SGOT and SGPT level in cholesterol induced hypercholesterolemia. Values are represented as mean±SEM, n=6. In statistical analysis, p<0.05 was considered to be significant; a = vs normal control; b = vs cholesterol control; p< 0.05 = *; p< 0.01= ^; p< 0.001= #.

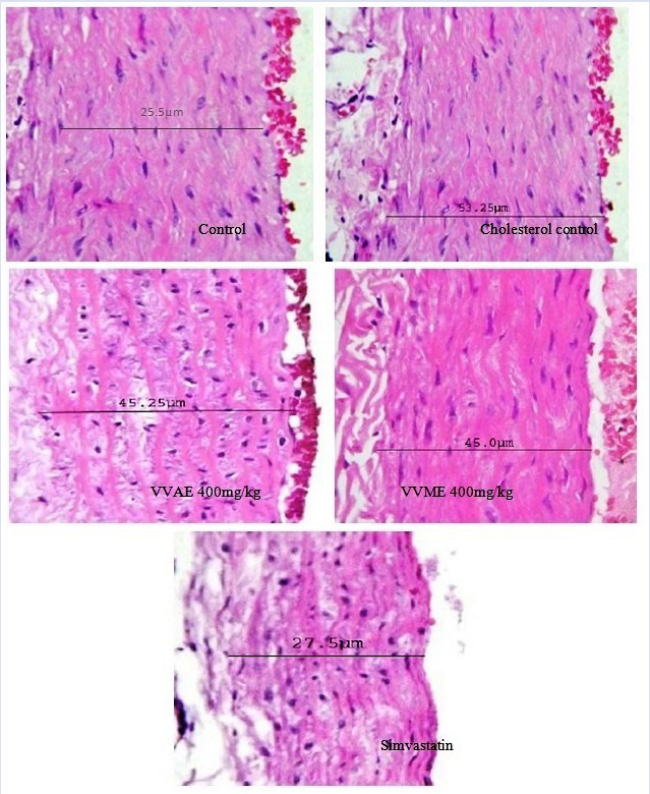

Figure 8: Histology of blood vessels (Tunica intima + media thickness) of different groups.

reports showed that cholesterol and high dietary fat induce hypercholesterolemia in animal models.[37,38] Similar results were also observed with the high cholesterol diet animals have increase lipid status and increased lipid level act as indicator of establishment of hypercholesterolemia in animal models.

HDL cholesterol is inversely connected with total cholesterol and several evidences are available regarding this fact. A reduction in HDL level may impair the clearance of cholesterol from the arterial wall and speed up the development of atherosclerosis that further lead to ischemic heart diseases.[39]

Effect of VVME and VVAE on SGOT and SGPT level

Administration of cholesterol diet resulted in elevation of SGOT and SGPT levels in cholesterol control animals as compared to normal control after 21 days. Administration of different doses of VVME and VVAE significantly attenuated the elevated SGOT and SGPT levels. (Figure 7) The elevated level in cholesterol control animals may be due to leakage of the enzymes into the serum and damage the integrity of the heart and liver. Also, increased level of these enzymes is reported as indicators of deliberate risk of cardiovascular disease.[40] In case of severe hepatocellular injury, SGOT and SGPT are released into serum. In the absence of viral hepatitis and alcoholism, increased SGPT level can lead to a higher risk of cardiovascular disease with more risk in women. Also a high SGOT content is found in heart which becomes more elevated in myocardial infarction case.[41] As well as, in the present study, histology results showed the disruption of endothelial lining in aorta, presence of foamy macrophage, increase thickness of lining and cardiovascular distress in cholesterol fed rats.

Histopathology of blood vessels

The histology of blood vessels (Tunica intima + media thickness) in cholesterol induced atherosclerosis is shown in8. In normal control section, the layers of artery and endothelial lining appeared intact and tunica intima, media and adventitia appeared within normal limits. The thickness of tunica intima + media was found to be 25.5 µm. In cholesterol control section, the layers of artery appeared intact except for disruption of the endothelial lining. Within the tunica intima and media were seen lipids containing elongated smooth muscle cells in single and aggregates of foamy macrophages. The tunica intima + media thickness was found to be 53.2 µm. In Atorvastatin section, the layers of artery and endothelial lining appeared intact. The tunica intima, media and adventitia appeared within normal limits. The thickness of tunica intima + media was found to be 27.5µm. In VVME 400 mg/kg section, the layers of artery appeared intact and few areas appeared disrupted. There were seen few scattered lipid containing spindle cells between the tunica intima and tunica media. Tunica adventitia appeared within normal limits and thickness was found to be 45.0 µm. In VVAE 400 mg/kg section, layers of artery appeared intact except for disruption of the endothelial lining. Within tunica intima and media were seen lipids containing smooth muscle cells in single and aggregates of foamy macrophages. The thickness of tunica intima + media was found to be 45.25 µm.

Effect on atherogenic index and % protection

The cholesterol control showed significant atherogenic index as compared to normal control (0.422 - high risk). In VVME 400 mg/kg found lowest atherogenic index (0.120) and maximum % protection as compared to other experimental groups. (Table 5) In 400 mg/kg of VVME group, atherogenic index and lipid profile were significantly improved with an improvement in the thickening of aortic walls. It can be state that decreased lipid levels might be an experimental tool to determine anti-atherogenicity of plant extract or other metabolites. As well as, histological assessment can be considered primarily to determine the degree of degeneration of atherosclerosis in biochemical markers.[42]

CONCLUSION

Grapes are utilised and grape skins and seeds produced in large quantities by the winemaking industry are increasingly used to obtain functional food ingredients.[1] Grapes are the better source of antioxidative constituents than skins of grape/wine byproducts. Functional ingredients of grape include several flavonoids with a phenolic nature such as monomeric flavanols, dimeric, trimeric and polymeric procyanidins,

and phenolic acids.[43,44] A few reports also indicated that extract of *Vitis vinifera* have strong antioxidant activity.[45] According to literature, flavonoids possess many pharmacological activities like antihyperlipidemic, hypoglycemic and antidiabetic activities. The presence of tannins and saponins in medicinal plant causes the inhibition of lipid absorption. So, it may be concluded that antioxidant and anti-hypercholesterolemic efficacy of *Vitis vinifera* might be due to presence of antioxidant property and active phytoconstituents.

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CONFLICT OF INTEREST

Authors have no conflict of interest

ABBREVIATION USED

AAI: Antioxidant activity index; **CVD:** Cardiovascular disease; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **DTNB:** 5,5'-Dithiobis, 2-nitrobenzoic acid; **EDTA:** Ethylenediaminetetraacetic acid; **GSH:** Glutathione; **GSSG :** Glutathione disulfide; **HDL:** High density lipoproteins; **LDL:** Low density lipoproteins; **NBT:** Nitro blue tetrazolium; **NO:** Nitric oxide; **ROS:** Reactive oxygen species; **SGOT:** Serum glutamic oxaloacetic transaminase; **SGPT:** Serum glutamic pyruvic transaminase; **SOD:** Superoxide Dismutase; **TCA:** Trichloroacetic acid; **VLDLL:** Very low density lipoproteins; **VVAE:** Vitis vinifera aqueous extract; **VVME:** Vitis vinifera methanolic extract.

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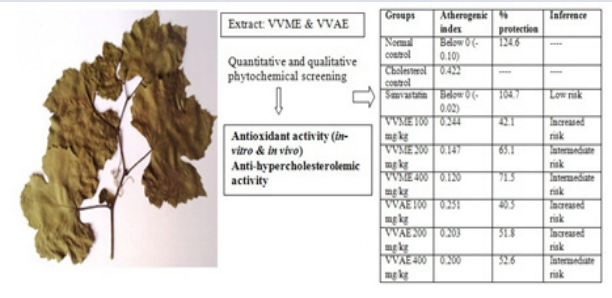
GRAPHICAL ABSTRACT



Groups	Atherogenic index	% protection	Inference
Normal control	Below 0 (-0.10)	124.6	----
Cholesterol control	0.422	----	----
Simvastatin	Below 0 (-0.02)	104.7	Low risk
VVME 100 mg/kg	0.244	42.1	Increased risk
VVME 200 mg/kg	0.147	65.1	Intermediate risk
VVME 400 mg/kg	0.120	71.5	Intermediate risk
VVAE 100 mg/kg	0.251	40.5	Increased risk
VVAE 200 mg/kg	0.203	51.8	Increased risk
VVAE 400 mg/kg	0.200	52.6	Intermediate risk

HIGHLIGHTS OF PAPER

- In methanolic extract, total tannins, total flavonoids and total phenolic contents were found in major amount.
- Different doses of extracts significantly attenuated the lipid levels and effectiveness was confirmed with histological results.
- VVME was found to be more effective as compared to VVAE.
- Antioxidant and anti-hypercholesterolemic efficacy of *Vitis vinifera* might be due to presence of antioxidant property and active phytoconstituents.

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