Ameliorative Effect of Red Grape Leaf Extract on Insulin Resistance: *In Vivo* And *In Silico* Studies

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Abstract

Background: The prevalence of insulin resistance and non-alcoholic fatty liver disease (NAFLD) necessitates exploring natural remedies. Grape leaf extract (GLE), containing resveratrol and other polyphenols, shows potential in managing these conditions. The study aimed to investigate the secondary metabolites present in grape leaf extract (GLE), especially resveratrol. Method: Red grape leaves were collected from South Sinai, Egypt, and subjected to ultra-sonication for polyphenol extraction. High-performance liquid chromatography (HPLC) identified six polyphenols, with resveratrol being predominant. Thirty male Sprague Dawley rats were fed a high-fat diet to induce NAFLD. Rats were divided into groups, including GLE-treated and control groups. Biochemical parameters were measured to assess GLE's impact. Molecular docking was performed to understand resveratrol's mechanism of action on insulin resistance and liver dysfunction. Results: HPLC revealed significant levels of resveratrol in GLE. GLE (50%) exhibited control over blood glucose (mg/dl) (94.69 ± 3.16), insulin levels (µIU/ml) (16.80 ± 2.5), and improved insulin resistance HOMA-IR (3.9 ± 1.19). It also lowered liver enzymes such as ALT (45.20 ± 3.31), AST (59.60 ± 2.40), and ALP (335.10 ± 11.01 U/L) and improved lipid profiles (mg/dl), including cholesterol (90±10.2), triglycerides (66.21±4.0), HDL (40.10±3.5), and LDL (50.15±8.0). Molecular docking showed resveratrol's affinity for SIRT-1 and phosphodiesterases, indicating its role in regulating insulin resistance and liver function. Conclusion: GLE, particularly resveratrol, demonstrated synergistic effects in improving insulin resistance and hyperlipidemic liver in rats. It also exhibited hepatoprotective properties. Molecular docking highlighted resveratrol's potential mechanisms, making it a promising therapeutic candidate for managing insulin resistance and NAFLD.

Keywords: Red Grape Leaf Extract, Insulin Resistance, Non-Alcoholic Fatty Liver Disease, Situiin-1, Molecular Docking.

Significance | Red grape leaf extract contains polyphenols, particularly resveratrol, which can potentially control insulin resistance and non-alcoholic fatty liver disease.

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Editor Mohamed Khadeer Ahamed Basheer, And accepted by the Editorial Board May 10, 2024 (received for review Mar 31, 2024)

1. Introduction

Free radicals can harm biomolecules in the body, leading to oxidative stress. Antioxidants act as a defense mechanism against free radicals and their harmful effects, ultimately aiding in the prevention of multiples diseases. Metabolic syndrome is a combination of chronic inflammation, hypertension, insulin resistance, high triglyceride levels, and low HDL (high-density lipoprotein) cholesterol levels as in non-alcoholic fatty liver disease (NAFLD). These factors can increase the risk of type 2 diabetes and cardiovascular disease (Ciulca et al., 2021; Frum et al., 2022; Tiwari, 2015).

Insulin resistance (IR) is a metabolic disorder that is associated with an increased risk of developing multiple diseases such as fatty liver or non-alcoholic fatty liver diseases (NASFLD). The rising incidence of IR can be attributed to various lifestyle choices. IR is

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Please cite this article.
Naglaa M. Hamdy, Hanan M. El-Tantawy et al. (2024). Ameliorative Effect of Red Grape Leaf Extract on Insulin Resistance: In Vivo And In Silico Studies, Journal of Angiotherapy, 8(5), 1-13, 9673

characterized by a reduced response of insulin target cells, including skeletal muscle, adipose tissue, and liver, to normal levels of insulin, leading to impaired glucose metabolism. The occurrence of insulin resistance (IR) results in the elevation of blood sugar levels, which subsequently prompts compensatory hyperinsulinemia and a decline in the expression of glucose transporter type 4 (GLUT4) in the cell membrane. Prolonged consumption of a high-sugar diet poses a risk for developing IR. As a result, non-fat cells such as the skeletal muscle, liver, heart, and pancreas may accumulate plasma free fatty acids (FFAs) (El Agamy & Ahmed, 2020).

NAFLD is a metabolic disorder that can occur due to the accumulation of lipids in hepatocytes, in the form of free fatty acids (FFA) and triglycerides (TG). This accumulation can cause non-alcoholic steatohepatitis (NASH). The progression of non-alcoholic fatty liver disease (NAFLD) often leads to the development of NASH. In NAFLD, excess lipids accumulate in hepatocytes in the form of free fatty acids (FFA) and triglycerides (TG), which cause the formation of lipid droplets in hepatocytes. This leads to an increase in liver weight of more than 5% without alcohol consumption and causes hepatic steatosis, inflammation, and fibrosis. Ultimately, this can lead to the development of hepatocellular carcinoma (HCC). The imbalance of lipid metabolism occurs due to issues with the uptake, metabolism, export, and oxidation of fatty acids in hepatocytes (Sodum et al., 2022).

Non-alcoholic fatty liver disease (NAFLD) progresses to steatohepatitis due to two reasons. The first reason is associated with metabolic disorders such as obesity, insulin resistance, and diabetes mellitus, which are risk factors for non-alcoholic steatohepatitis (NASH). The second reason is caused by oxidative stress, inflammation, lipid peroxidation, pro-inflammatory cytokines, immune system response, gut, adipose tissue-derived factors, and genetic alteration, which occur due to hepatocyte injury and fibrosis. Understanding the two-reasons and how to deal with them is vital for developing effective treatment strategies for NASH (Abdelmalek, Abd-Elhamid, Abd Ellah, & Ismail, 2023; El Agamy & Ahmed, 2020; Sodum et al., 2022).

Metformin, which is also sold under the brand name Glucophage (approved by FDA, 1994), is an oral hypoglycemic medication that is often recommended as the initial treatment for type 2 diabetes. It is especially beneficial for patients who are overweight or obese. Metformin is also utilized to treat polycystic ovary syndrome (Blonde, Dipp, & Cadena, 2018). One of the major benefits of Metformin is that it does not cause weight gain and can be conveniently taken orally. Metformin is extremely effective in regulating blood glucose levels. It works by reducing the liver's glucose production and decreasing the absorption of glucose from food in the body. Furthermore, it enhances the body's sensitivity to insulin, a hormone that plays a key role in keeping blood sugar levels in check (Corcoran & Jacobs, 2018). In this study, the effect of red grape leaf extract in regulating insulin resistance was compared to that of Metformin, a standard drug used to control insulin resistance.

Incorporating natural products into a healthy diet can play a crucial role in preventing metabolic syndrome and reducing the risk of cardiovascular disease. For example, the consumption of natural polyphenols reduces the risk of several types of diseases including metabolic disorders. The red grape (*Vitis vinifera* L.) is renowned for its nutritional and pharmaceutical benefits, especially in its peel, leaf, and seed extracts. The winery and grape juice industries generates grape waste products that contain numerous of essential nutrients, such as lipids, proteins, carbohydrates, and polyphenols (Li et al., 2014). Producing new dietary supplements from these waste products aligns with our environmental goals and promotes sustainability.

Resveratrol is a natural substance with incredible health benefits. It has antioxidant properties that make it effective in preventing and treating diseases caused by oxidative stress. Numerous studies have shown the therapeutic value of resveratrol in reducing inflammation, regulating blood sugar levels, fighting cancer, and protecting the heart. Additionally, it is an effective anti-obesity agent that helps to improve lipid and glucose metabolism, thereby improving overall health. Resveratrol is found in various sources, but the highest concentration is found in grape especially in peels, making red wine a great source of this beneficial substance. Its phenolic hydroxyl groups play a vital role in its natural antioxidant and free radical scavenger properties, making it an essential tool in maintaining good health (Gong, Guo, & Zou, 2020; Reda et al., 2022). In this study, according to these previous reviews, red grape leaf extract was compared with synthetic resveratrol as a regulator of insulin resistance.Sirtuins are enzymes which require NAD as a cofactor and are a type of endogenous biological agents. They are categorized into seven types, known as SIRT-1 to SIRT-7. SIRT-1 has anti-inflammatory characteristics and enhances both insulin sensitivity and secretion. Recent investigations suggest a correlation between reduced levels of SIRT-1 gene expression and the development of NAFLD through the NOTCH signaling pathway. This pathway is considered a molecular pathway that plays a crucial role in various biological processes, such as adipogenesis. Resveratrol has been shown to stimulate SIRT-1 in *in vivo* conditions, leading to the deacetylation of the NOTCH-1 receptor and consequently inhibiting NOTCH-1 activity (Sodum et al., 2022). The phosphodiesterases (PDEs) enzyme family is vital in preserving the proper levels of cyclic adenosine monophosphate (cAMP) within cells, which is crucial in regulating cellular inflammatory pathways. Recent studies have indicated that PDE inhibitors could provide therapeutic benefits for a range of inflammatory disorders (Muhammad, Elwai, & Abd El Rahman,

2019), including insulin resistance and NAFLD. These inhibitors work by preventing cGMP degradation and could offer a new and innovative therapeutic strategy for treating metabolic diseases. Resveratrol has been found to be effective in treating NAFLD by partially inducing autophagy through the PDE-SIRT1 signaling pathway. This new evidence suggests that Resveratrol can improve hepatic steatosis, which is the abnormal accumulation of fat in the liver, and highlights its potential as a therapeutic option for NAFLD treatment (Y. Zhang et al., 2015).

The current study seek to assess the impact of red grape leaf extract, in comparison to resveratrol and metformin as standards, on insulin resistance linked to non-alcoholic fatty liver disease. The study aims to estimate the enhancement of insulin resistance and liver biomarkers *in vivo*, as well as assess the mechanisms of resveratrol, as one of the most effective phenol in grape leaf extract, against SIRT-1 and PDE target sites in safeguarding the liver from the consequences of metabolic disorders.

2. Material and Methods

The red grape leaves (*Vitis vinifera*) were obtained from grape farms, South Sinai, Egypt. Ultrasonic extraction was carried out in Technological Incubator for agricultural nanotechnology, Desert Research Center (DRC), Cairo, Egypt. *In vivo* study was done in Global Lab, Egypt. HPLC analysis was carried out in The Regional Center for Mycology and Biotechnology, Al-Azhar Uni., Egypt. The chemical and standards were purchased from Sigma Aldrich, St. Louis, MO, USA.

2.1. Dried red grape leaf powder preparation and extraction

500g the grape leaves were washed, and dried in shaded place, grounded, and sieved to obtain particles with the size between 425 and 125 μm. The dried grape leaf sample was packaged in dark glass bottle and stored at −20°C in darkness for further analysis. To extract the contents 2g with 100ml of 80% ethanol was stirred by a magnetic stirrer for 5 minutes. The extraction process was then carried out in a 10-liter sonication water bath using ultrasonic assistance. The frequency was set at 40 KHz and the temperature and extraction time were adjusted from the control panel. Once the extraction was complete, the flask was cooled with cool water to room temperature. The solution was then filtered through filter paper No. 5A under vacuum, and the resulting solution was collected in a volumetric flask. Finally, the solution was utilized to determine the total phenolic compounds, antioxidants, and anthocyanin contents (Ghafoor & Choi, 2009).

2.2. HPLC determinations for dried grape leaf extract phenolic compounds

HPLC determinations of phenolic compounds in dried grape pulp extract were assessed by HPLC as described by (Abo El-Fadl, Osman, Al-Zohairy, Dahab, & Abo El Kheir, 2020). The Eclipse plus C18 column (4.6250 mm, i.d. 5 m) was used for the separation. At a flow rate of 1 mL/min, the mobile phase was composed of water (A) and 0.02% tri-floro-acetic acid in acetonitrile (B). The mobile phase was programmed in the following order: 0 min (80% A), 0-5 min (80% A), 5-8 min (40% A), 8-12 min (50% A), 12-14 min (80% A), and 14-16 min (80% A). At 280 nm, the multi-wavelength detector was monitored. The injection volume was ten microliters (series 1200). The column temperature was kept constant at 35 °C. Table 1 represents the HPLC results. Trans-resveratrol was the most active compound.

2.3. *In vivo* study

Thirty male Sprague-Dawley albino rats, weighing 150 g, at the beginning of the study, were housed in optimal conditions [plastic cadges, temperature of 25±1°C, cycles of light /darkness that lasted 12 hours]. The experiment duration was 45 days, and the changes in body weight were recorded weekly. The rats were divided following:

A] Normo-lipidemic (NC-Lip) group / 5 rats: fed on a basal diet as described in Table 1.

B] Hyperlipidemic group (HLip) /25 rats: fed on a basal diet with 5% cholesterol, which subsequently divided into five subgroups:

1] Positive control-HLip (PC-HLip) / 5 rats: fed on a basal diet with 5% cholesterol only.

2] Hyperlipidemic- grape extract treated groups (HLip-GLE)/ 5 rats: fed on a basal diet, 5% cholesterol, and 25% dried grape leaf extract.

3] Hyperlipidemic- grape extract treated groups (HLip-GLE) / 5 rats: fed on a basal diet, 5% cholesterol, and 50% dried grape leaf extract.

4] Hyperlipidemic- resveratrol standard treated groups (HLip-RES) / 5 rats: fed on a basal diet, 5% cholesterol and 25% of resveratrol standard.

5] Hyperlipidemic- metformin standard treated groups (HLip-MET) / 5 rats: fed on a basal diet, 5% cholesterol and 25% of metformin standard.

2.4. Determination of biochemical profiles

Blood samples were taken from the tail vein with a disposable plastic syringe. Serum was obtained by centrifugation at 1500 rpm for 15 min at suitable temperature.

For hemostatic glucose parameters: Serum glucose was measured using commercially available glucometer and strips (Accu-Chek Sugar Meter Monitoring System, Roche Diabetes Care India Pvt. Ltd., India). Serum insulin was determined using ready-to-use Rat ELISA kits from Biospes (Chongqing, China). Finally, homeostasis model assessment of insulin resistance (HOMA-IR) was evaluated using the following equation:

R = glucose concentration mmol/l × insulin μIU/ml/ 22.5

For liver enzymes and lipids profile: Alanine aminotransferase [ALT] and Aspartate Aminotransferase [AST] levels were measured enzymatically using commercially available assay kits.

Plasma was collected into heparinized tubes, and centrifuged at 2500 rpm for 10 min at 4C° temperature for triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) levels were measured enzymatically using commercially available assay kits. All the samples were stored under -20 °C for further analysis.

2.5. Molecular docking study

Computational techniques are commonly used in drug design and drug discovery to perform virtual biological screening. Recent applications of computational chemistry have improved our understanding of targeted sites and helped identify compounds as inhibitors or activators. In this study molecular docking was designed according to Biswas et al. 2013 (Biswas, Balac, Narlakanti, Haque, & Hassan, 2013), resveratrol's possible activity against SIRT-1 and PDE target sites as hepatoprotective agents was investigated using the Auto-Dock Vina. The target proteins were downloaded from the protein data bank (protein Id: 5BTR-4MD6). The protein structure and resveratrol were prepared, and energy was minimized using an MMFF94 force field. Molecular docking was performed, generating twenty poses, from which the best orientations were selected based on affinity scores and RMSD values.

2.6. Statistical analysis

Data were analyzed by (ANOVA) test, SAS (1999) statistical package of the general linear model (GLM). The average was based on three replicates ($p \leq 0.05$) (SAS Statistical Analysis System, 1999) (Petruccelli, Nandram, & Chen, 1999).

3. Results and Discussion

3.1. Dried red grape leaf powder extraction and HPLC determinations

In the current study, the extraction was done using ultrasound-assisted extraction (UAE), which is one of the non-conventional extraction methods. This study uses ethanol as the chosen solvent for UAE. Red grape leaves, scientifically known as *Vitis vinifera*, are a by-product of the processing of grapes. These leaves are usually disposed of as agricultural waste. To promote sustainable agriculture and enhance food systems, it is important to explore the nutritional values of these by-products. Red grape leaves contain several bioactive compounds (mg/kg) according to standards used as represented in table (2), including resveratrol (15.36 ± 0.34), quercetin (13.90 ± 0.73), caffeic acid (4.5± 0.57), kaempferol (2.37 ± 0.57), gallic acid (11.73 ± 0.51), and ferulic acid (1.22 ± 0.34) which have pharmacological effects on human health. These compounds exhibit antioxidant, anti-inflammatory, anti-obesity, anti-diabetic, and hepatoprotective properties. Additionally, grape leaves extract can be used as a functional ingredient in the production of both food and non-food products (Singh et al., 2023). This study investigated the anti-diabetic effects of red grape leaf extract with pre-diabetic and diabetic type 2 conditions, particularly in relation to NASH.

3.2. Effect of dried grape leaf extract on weight gain/ obesity

The data denoted in Table 3, indicate the impact of dried grape leaf extract on weight gain or obesity in a hyperlipidemic rat model. The group of rats fed on a basal diet+ dried grape pulp extract (HLip-GE) showed a significant decrease ($p \leq 0.05$) among all concentrations used during the experiment duration. The 50% dried grape leaf extract concentration was recorded (153.9±4.9) in the first week, while resveratrol standard was recorded (149.4± 9.7). In the second week the 50% dried grape leaf extract concentration was recorded (171.6 ± 10.3), and resveratrol standard was recorded (169.2 ± 9.3). The data demonstrated that the 50% dried grape leaf extract concentration showed most significant decrease in weight gain when compared to the control groups.

Additionally, there was a significant decrease ($p \leq 0.05$) in the relative weight of liver organs at the end of the experiment, especially with the 50% dried grape leaf extract concentration (3.1±0.55), when compared to resveratrol standard and the control groups. These results with agree with a previous study investigated the action of resveratrol on NAFLD in an animal model of a high carbohydrate-fat diet, which resveratrol (10 mg/day) reduces liver fat deposition in the treated group compared to the controls (Bujanda et al., 2008). However, metformin was excluded from the table due to its known effect in reducing weight gain.

3.3. Effect of dried grape leaf extract on blood glucose and insulin resistance

According to the study, the blood glucose level was markedly higher in the PC-HLip group (130 ± 4.01 mg/dl) in comparison to the NC-Lip group (84.2 ± 6.3 mg/dl) ($P < 0.05$). Notably, both groups of HLip-GLE (25% and 50%) demonstrated a direct correlation between the reduction in blood glucose level and an increasing concentration. These findings suggest that the HLip-GLE groups could be of value in reducing blood glucose levels (110 ± 3.9, and 100.2 ± 3.7 mg/dl respectively), which the 50% concentration displaying a more significant effect. However, HLip-RES and HLip-MET groups both exhibited a considerable reduction in blood glucose levels (94.69 ± 3.16, and 89.4 ± 4.7 mg/dl respectively) to a certain extent, which was significantly lower than the PC-HLip group ($P < 0.05$), yet relatively higher than NC-Lip group ($P < 0.05$) as represented in Table 4.

The PC-HLip group exhibited a significantly higher serum insulin level (28.20 ± 3.4 µIU/ml) compared to the control group (14.3 ± 2.12 µIU/ml) ($P<0.05$). Conversely, the groups of HLip-GLE (25% and 50%), HLip-RES and HLip-MET groups demonstrated a significantly lower serum insulin level (24.4 ± 3.6, 20.2 ± 3.8, 16.80 ± 2.5, and 15.7 ± 1.6 µIU/ml respectively) than the PC-HLip group ($P<0.05$). Notably, the serum insulin levels in groups of

HLip-RES (16.80 ± 2.5 μIU/ml) and HLip-MET (15.7 ± 1.6 μIU/ml) remained significantly higher than those in the control group.

Moreover, the calculation of the HOMA-IR index, that indicated insulin resistance, significantly increased in the PC-HLip group (9.0 ± 1.4) compared to the control group (2.9 ± 0.04) (*P<0.05*). However, the HOMA-IR index significantly decreased among HLip-GLE groups (6.8 ± 1.2 for 25%, and 4.9 ± 1.01 for 50% red grape leaf extract). Finally, the HLip-RES (3.9 ± 1.19) and HLip-MET (3.4 ± 0.6) groups significantly lower than the PC-HLip group (*P<0.05*), and were demonstrated a slight significant change compared to the control group as referred to Table 4.

3.4. Effect of dried grape leaf extract on blood lipid profile

According to the study, the PC-HLip group which consumed a high fat diet (HFD) had noticeably higher levels of serum cholesterol (124±9 mg/dl) than the control group (88±5.2 mg/dl) (*P<0.05*). However, providing resveratrol or metformin standard groups significantly reduced serum cholesterol levels (90±10.2 and 89±12.3 mg/dl, respectively). Although cholesterol levels were still significantly high in HLip-GLE 25% (105±14 mg/dl), and 50% (96±12 mg/dl) groups than the control group (*P<0.05*), they were considerably lower than those of the PC-HLip group as in Table 5.

The study found that the PC-HLip group had significantly higher serum triglycerides levels (81.5±7.11 mg/dl) than the control group (62.6±2.5 mg/dl). However, the resveratrol and metformin standards groups exhibited a significant reduction in serum triglycerides levels (64.21±4, and 62.3±4.4 mg/dl, respectively) compared to the PC-HLip group (*P<0.05*). Furthermore, this group showed no significant difference in comparison to the corresponding level in the control group. When compared to the corresponding level in the control group, both groups of HLip-GLE (25%, and 50%) showed a significant change in serum triglycerides levels (70.3±4.4, and 68.31mg/dl, respectively) as evidenced in Table 5.

The results of the study showed that the PC-HLip group had a considerable increase in serum LDL (78.9±6.7 mg/dl) when compared to the control group (44.75±6.9 mg/dl) (*P<0.05*). However, both the HLip-RES and HLip-MET groups demonstrated a significant decrease in serum LDL, reaching levels that were significantly lower than the PC-HLip group (50.15±8 and 47.3±7.7, respectively), but HLip-MET group recorded no significant difference when compared to the control group (*P<0.05*). In the case of the HLip-GLE (25% and 50%) groups, there were a significantly lower level of LDL compared to the PC-HLip group, but still significantly higher than the control group (66.13±7.7and 56.25±8 mg/dl, respectively) (*P<0.05*) as described in Table 5.

The study found that the level of serum PC-HLip group (27±4.90 mg/dl) was significantly lower than in the control group (*P<0.05*). However, the HLip-GLE (25% and 50%) groups showed a significantly higher level of serum HDL (36.25±5.4, and 39.30±5 respectively) compared to the PC-HLip group. On the other hand, the HLip-RES and HLip-MET groups exhibited a significantly higher level of HDL than the PC-HLip group (*P<0.05*) and had an insignificant change compared to the control group (42.5±6.0) as shown in Table 5.

3.5. Effect of dried grape leaf extract on serum liver function profile

Based on the findings, the groups treated with HLip-RES and HLip-MET experienced a notable reduction in liver enzymes (ALT 45.02 ± 2.5 and 44.01 ± 2.5 U/L, AST 45.02 ± 2.5 and 44.01 ± 2.5 U/L, and ALP 334.1±11.6 and 332.5 ± 14.6 U/L, respectively) compared to the high-fat diet group (PC-HLip group: ALT 61.20 ± 3.20 U/L, AST 78.80 ± 2.20 U/L, and ALP 426.75 ± 14.7 U/L). The liver enzyme levels returned to nearly normal levels comparable to those of the control group, as detailed in Table 6. Even the HLip-GLE groups at 25% and 50% showed significantly lower liver enzyme levels (ALT 50.20 ± 3.31 and 47.10 ±3.94 U/L, AST 59.60 ± 2.40 and 56.40 ±3.20 U/L, and ALP 340.10 ± 11.1and 338.2 ± 11.63 U/L, respectively) than the PC-HLip group, remaining close to control group values (ALT 44.03 ± 2.25 U/L, AST 54.80 ± 2.21 U/L, and ALP 330.2 ± 18.63 U/L), as presented in Table 6.

3.6. Resveratrol - SIRT-1 and PDE molecular docking

The study used a computer-based chemistry approach to assess Resveratrol's potential activity against the SIRT-1 target site. Table 7 shows the affinity scores and root-mean-square deviation (RMSD) values collected during the process. According to the analysis, Resveratrol exhibited an energy binding of -6.93 kcal/mol against the SIRT-1 target site. The binding was achieved through three hydrophobic interactions with Pro212, Phe414 and Val445. Additionally, Resveratrol formed three hydrogen bonds with Asp292, Asp298, and Lys444 with bond lengths of 1.67, 1.49, and 1.74 Å (fig. 1). Resveratrol binding mode displayed an energy binding of -5.42 kcal/mol against the PDE target site. It formed eight hydrophobic interactions with Leu725, His613, Asp764, Ala767, Tyr612, Ile768, Phe820, and Val782. Additionally, Resveratrol interacted with Asp654, Gln817, His617, and His653 through four hydrogen bonds with bond lengths of 3.09, 2.18, 2.42, and 2.92 Å as shown in Figure 1.

4. Discussion

This study evaluated the potential benefits of red grape leaf extract's phenolic compounds, particularly resveratrol, in addressing overweight, hyperlipidemia, insulin resistance, and liver enzyme functions commonly associated with metabolic syndrome.

Throughout history, natural products were the primary option for preventing and curing human illnesses. These remedies have served as valuable resources for drug discovery, but the bioactive compounds they contain are usually present in low quantities.

Table 1. Basal diet ingredients

Ingredient	(%)
Wheat flour	50.00
Wheat bran	19.00
Rice powder	11.25
Egg white	10.00
Casein	8.00
Soya bean oil	1.00
Table salt	0.50
Mixture of vitamins	0.125
Mixture of menials	0.125

Table 2. HPLC active compounds identification in dried grape leaf [GLE] extract

Polyphenolic compounds of dried-GLE	**Quantity (mg/kg)**
Trans-resveratrol	15.36 ± 0.34
Ferulic acid	1.22 ± 0.34
Gallic acid	11.73 ± 0.51
Kaempferol	2.37 ± 0.57
Quercetin	13.90 ± 0.73
Caffeic acid	4.5± 0.57

Table 3. Effect of oral administration of dried grape leaf extract at different concentrations on body weight gain and relative weight of the liver organs *in vivo*

Parameters	**NC-Lip**	**PC-HLip**	**Treated groups (%) per day**		
			HLip-GLE 25%	**HLip-GLE 50%**	**HLip-RES 25%**
Body weight gain					
Frist week	149.8± 9.9 ^{b}	163.5^{a}±4.6	159.2^{a}±4.8	153.9^{ab}±4.9	149.4^{b}± 9.7
Second week	170.7 ± 10.5 ^{b}	187.1 ± 11.5 ^{a}	178.4 ± 11.2^{ab}	171.6 ± 10.3 ^{b}	169.2^{c} ± 9.3
Relative weight of liver organs (final) [body weight%]					
Liver	3.33^{b}±0.59	4.5^{a}±1.29	3.4^{b}±0.6	3.1^{b}±0.55	2.95^{c}±0.5

NC-Lip: *non-lipidemic group,* ***PC-HLip:*** *hyperlipidemic positive control group,* ***HLip-GLE:*** *hyperlipidemic group treated with dried grape leaf extract.* ***HLip-RES:*** *hyperlipidemic group treated with resveratrol standard,* ***HLip-MET:*** *Hyperlipidemic group treated with metformin standard. Values represent the mean ± SD, Values in the same row that do not share a common superscript are significantly different at p ≤ 0.05.*

Table 4. Effect of [GLE] on the hemostatic glucose parameters in HFD-induced fatty liver rats

Parameters	Investigated groups					
	NC-Lip	**PC-HLip**	**HLip-GLE 25%**	**HLip-GLE 50%**	**HLip-RES 25%**	**HLip-MET 25%**
Glucose (mg/dl)	84.2 ± 6.3^{d}	130 ± 4.01^{a}	110 ± 3.9^{b}	100.2 ± 3.7^{c}	94.69 ± 3.16^{cd}	89.4 ± 4.7^{d}
Serum Insulin (μIU/ml)	14.3 ± 2.12^{d}	28.20 ± 3.4^{a}	24.4 ± 3.6^{b}	20.2 ± 3.8^{c}	16.80 ± 2.5^{cd}	15.7 ± 1.6^{cd}
HOMA-IR	2.9 ± 0.04^{d}	9.0 ± 1.4^{a}	6.8 ± 1.2^{b}	4.9 ± 1.01^{c}	3.9 ± 1.19^{cd}	3.4 ± 0.6^{cb}

NC-Lip: *non-lipidemic group,* ***PC-HLip:*** *hyperlipidemic positive control group,* ***HLip-GE:*** *hyperlipidemic group treated with dried grape leaf extract.* ***HLip-RES:*** *hyperlipidemic group treated with resveratrol standard,* ***HLip-MET:*** *Hyperlipidemic group treated with metformin standard,* ***HOMA-IR:*** *Homeostatic model assessment of insulin resistance. Values represent the mean ± SD, Values in the same row that do not share a common superscript are significantly different at p ≤ 0.05.*

Table 5. Effect of [GLE] on the blood lipid profile in HFD-induced fatty liver rats

Parameters	Investigated groups					
	NC-Lip	**PC-HLip**	**HLip-GLE 25%**	**HLip-GLE 50%**	**HLip-RES 25%**	**HLip-MET 25%**
Serum Cholesterol (mg/dl)	88±5.2^{d}	124±9.0^{a}	105±14^{b}	96±12^{c}	90±10.2^{cd}	89±12.3^{d}
Serum Triglycerides (mg/dl)	62.6±2.5^{d}	81.5±7.11^{a}	70.3±4.4^{b}	68.31±4.4^{c}	64.21±4.0^{d}	62.3±4.4^{d}
Serum LDL (mg/dl)	44.75±6.9^{d}	78.9±6.70^{a}	66.13±7.7^{b}	56.25±8^{c}	50.15±8.0^{cd}	47.3±7.7^{d}
Serum HDL (mg/dl)	42.5±6.0^{a}	27±4.90^{d}	36.25±5.4^{ab}	39.30±5^{b}	40.10±3.5^{ab}	41.45±5.4^{a}

NC-Lip: *non-lipidemic group,* ***PC-HLip:*** *hyperlipidemic positive control group,* ***HLip-GE:*** *hyperlipidemic group treated with dried grape leaf extract.* ***HLip-RES:*** *hyperlipidemic group treated with resveratrol standard,* ***HLip-MET:*** *Hyperlipidemic group treated with metformin standard. Values represent the mean ± SD, Values in the same row that do not share a common superscript are significantly different at* $p \leq 0.05$.

Table 6. Effect of [GLE] on the serum liver function indicators in HFD-induced fatty liver rats

Parameters	Investigated groups					
	NC-Lip	**PC-HLip**	**HLip-GLE 25%**	**HLip-GLE 50%**	**HLip-RES 25%**	**HLip-MET 25%**
ALT (U/L)	**44.03 ± 2.25^{d}**	**61.20 ± 3.20^{a}**	**50.20 ± 3.31^{b}**	**47.10 ±3.94^{c}**	**45.02 ± 2.5^{cd}**	**44.01 ± 2.5^{d}**
AST (U/L)	**54.80 ± 2.21^{d}**	**78.80 ± 2.20^{a}**	**59.60 ± 2.40^{b}**	**56.40 ±3.20^{c}**	**55.10 ±2.2^{cd}**	**54.40 ±3.2^{d}**
ALP (U/L)	**330.2 ± 18.63^{d}**	**426.75 ± 14.7^{a}**	**340.10 ± 11.1^{b}**	**338.2 ± 11.63^{c}**	**334.1±11.6^{cd}**	**332.5 ± 14.6^{cb}**

NC-Lip: *non-lipidemic group,* ***PC-HLip:*** *hyperlipidemic positive control group,* ***HLip-GE:*** *hyperlipidemic group treated with dried grape leaf extract.* ***HLip-RES:*** *hyperlipidemic group treated with resveratrol standard,* ***HLip-MET:*** *Hyperlipidemic group treated with metformin standard. Values represent the mean ± SD, Values in the same row that do not share a common superscript are significantly different at* $p \leq 0.05$.

Table 7. Molecular docking analysis of Resveratrol against SIRT-1 and PDE *target site.*

Targets	**Tested compounds**	**RMSD value (Å)**	**Docking (Affinity) score (kcal/mol)**	**Interactions**	
				H.B	**Pi –interaction**
SIRT-1	**Resveratrol**	1.32	-6.93	3	3
PDE	**Resveratrol**	1.75	-5.42	4	8

SIRT-1: *Siturin-1,* ***PDE:*** *Phosphordiesterases,* ***H.B:*** *hydrogen bond,* ***RMSD:*** *root mean square deviation*

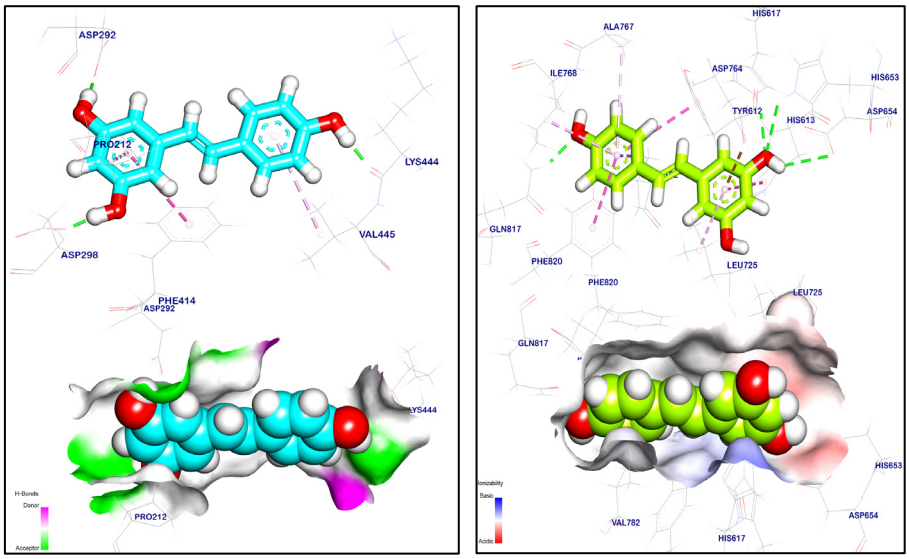

Figure 1. 3D orientation and surface mapping of Resveratrol against SIRT-1 target site (left). And 3D orientation and surface mapping of resveratrol against PDE target site (right).

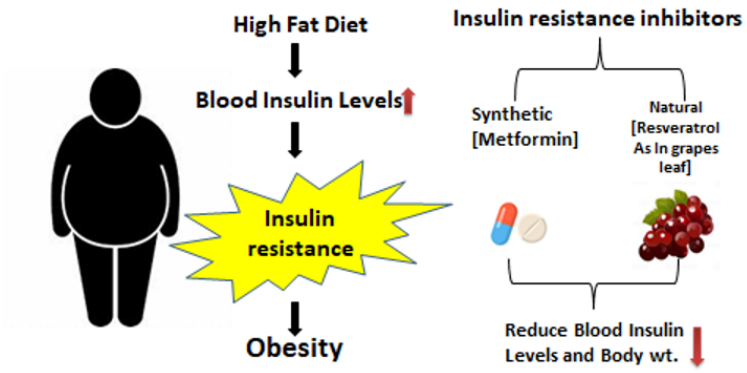

Figure 2. Insulin resistance; reasons and managements

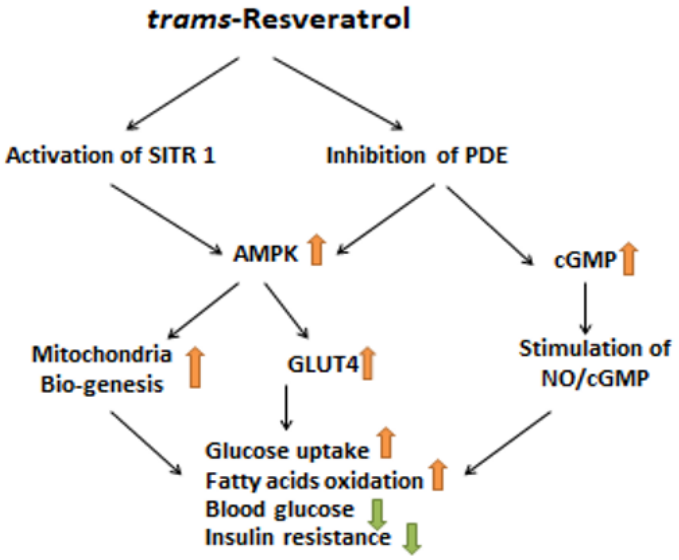

Figure 3. Mechanisms by which resveratrol may counteract insulin resistance.

Hence, it is vital to establish efficient and specific techniques for extracting and purifying these bioactive compounds in modern medicinal industry (Q.-W. Zhang, Lin, & Ye, 2018). Extraction is a crucial stage between analytical processes and product production. Its main objective is to recover desired components from a sample matrix while minimizing contaminants. Secondary metabolites, known as polyphenols, such as found in red grape leaves have multiple biological benefits. The appropriate extraction technique depends on various factors such as the chemical makeup, particle size, and the presence of interfering compounds. Extraction techniques can either be conventional or non-conventional (Lefebvre, Destandau, and Lesellier, 2021). Several alternative techniques are available for extracting molecules and biomaterials from red grape leaves. Among these, ultrasound-assisted extraction (UAE) is a popular and cost-effective method that uses sound waves to accelerate the extraction process. Environmentally-friendly extraction methods, such as green extraction, are preferred since they reduce the health risks associated with toxic solvents (Tiwari, 2015).

Numerous studies have emphasized the importance of natural antioxidants, specifically plant polyphenols. These compounds have anti-inflammatory and anti-atherogenic properties, and they can reduce oxidative stress in cells and increase metabolic rate. Therefore, they have the potential to be used as anti-obesity agents. The study we are discussing takes a detailed look at the phenols present in the red grape leaf extract, ranging from the lowest to the highest.

Ferulic acid, which had the lowest value in our extract (1.22 ± 0.34 mg/kg), is known to be non-toxic and possess many physiological functions, such as anti-inflammatory, antioxidant, antimicrobial, anticancer, and antidiabetic effects. It has been widely used in the pharmaceutical, food, and cosmetics industries. A recent study conducted in 2024 investigated the potential of ferulic acid in suppressing TNF-α-treated inflammation and insulin resistance in adipocytes, which yielded promising results (Park & Han, 2024).

Kaempferol (2.37 ± 0.57 mg/kg), a flavonoid found in the current extract of red grape leaf, has ability to regulate hepatic gluconeogenesis and blood glucose homeostasis in high-fat diet, which a previous study found that Kaempferol, a natural flavonoid, can regulate hepatic gluconeogenesis and maintain blood glucose homeostasis in high-fat diets. This anti-diabetic compound enhances insulin sensitivity, inhibits glucose production, and suppresses the enzymatic activity of pyruvate carboxylase. These effects were observed in obese mice and HepG2 cells without affecting body weight gain, food consumption, or adiposity. Thus, Kaempferol shows promise as a potential therapeutic agent for managing diabetes and other metabolic disorders (Alkhalidy et al., 2018).

Caffeic acid (4.5± 0.57 mg/kg), depending to a previous systematic review, the results collected from major electronic databases to evaluate the impact of caffeic acid (CA) and its derivatives on diabetes and its complications, revealed that caffeic acid and its derivatives have anti-oxidant, anti-inflammatory, and anti-diabetic properties. They reduce blood glucose levels, oxidative stress markers, and inflammatory cytokines while increasing anti-oxidative markers in cellular and animal models of diabetes. They promote insulin secretion and ameliorate insulin resistance. They also protect against diabetes complications (Akhlaghipour, Shad, Askari, Maharati, & Rahimi, 2023).

Gallic acid which recorded respectable value in this study (11.73 ± 0.51 mg/kg), was found to have an ameliorative effect on hypertriglyceridemia and fat accumulation in perirenal adipose tissues of high-fructose diet (HFD)-induced diabetic rats. This prevous study showed that gallic acid reduced plasma glucose and triglyceride (TG) levels in HFD rats and restored expression of insulin signaling-related proteins in the perirenal adipose tissues of HFD rats. Gallic acid may alleviate hypertriglyceridemia and fat accumulation through enhancing glycolysis and lipolysis pathways in perirenal adipose tissues of HFD rats, suggesting its potential in preventing the progression of diabetes mellitus (DM) complications (Huang, Chang, Yang, Wu, & Shen, 2018).

Quercetin also recorded high value (13.90 ± 0.73 mg/kg) in our study, has segnificant effect on insulin sensitivity recorded by several *in vitro* and *in vivo* studies. Results showed improved glycemic control, but impaired neuronal control of glucose homeostasis (Russo, Picconi, Malandrucco, & Frontoni, 2019). Further research is needed to understand quercetin's role in regulating glucose homeostasis in the central nervous system.

Resveratrol, the maestro of this investigation, recorded the highest respected value (15.36 ± 0.34 mg/kg). Resveratrol, or 3,5,4'-trihydroxytrans-stilbene, is a powerful active biomolecule naturally occurring within the polyphenol family. This non-flavonoid polyphenol is primarily present in nuts, grapes, and berries. Resveratrol has demonstrated antioxidant and anti-inflammatory effects, which are beneficial in managing metabolic syndrome, type 2 diabetes mellitus, and NAFLD. Furthermore, several studies suggest that Resveratrol regulates lipid metabolism and modulates apoptosis (Abdelmalek et al., 2023). Resveratrol supplementation may help stabilize diabetes by activating sirtuins and estrogen receptors to increase glucose uptake. It can also enhance vasodilator function, which may help to alleviate insulin resistance in type-2 diabetes mellitus. By improving blood perfusion of skeletal muscle, resveratrol can facilitate glucose delivery and utilization, improving insulin sensitivity (Wong & Howe, 2018). The study focus on the combined impact of polyphenols found in red grape leaves on weight gain, liver function, liver profile, and glucose levels. An *in silico* investigation will be undertaken to elucidate how resveratrol

generates its beneficial effects in regulating insulin resistance associated with NAFLD.

Obesity is widely recognized as a significant and established risk factor for NAFLD. NAFLD is a chronic metabolic disorder that affects liver histology and can lead to the development of a more severe liver condition known as non-alcoholic steatohepatitis (NASH). NASH alters fatty acid metabolism, causing the body to store TG and FFAs in liver cells. If untreated, NASH can lead to liver cancer. No drug has been approved exclusively for NASH treatment, despite the use of alternative drugs. NOTCH-1 receptors are highly expressed in individuals with NAFLD/NASH conditions. Studies have shown this overexpression leads to insulin resistance (Sodum et al., 2022). It's crucial to be aware of this information for better understanding and management of these metabolic disorders.

Resveratrol has beneficial properties crucial in preventing this metabolic condition and reducing liver inflammation (Xin et al., 2013). In our recent study, we discovered that a high-fat diet (HFD) caused significant obesity that was evident by the gradual increase in body weight after two weeks of consuming the diet. However, our results showed that resveratrol, which was also found in red grape leaf extract, could also decrease excessive body weight gain in a dose-dependent manner. In a previous study, the administration of resveratrol at a high dose of 400 mg/kg/day for 15 and 24 weeks significantly reduced weight gain in male C57BL/6J mice fed HFD (Reda et al., 2022). This result was support our investigation, which as a result, there may be a connection between the administration of high doses of resveratrol over a prolonged period and weight loss due to variation.

Consuming a high-fat diet has a negative impact on insulin sensitivity, which increases the risk of various diseases, including cardiovascular disease and type 2 diabetes. In this study, groups treated with resveratrol or metformin and red grape extract in concentrations of 25% and 50% showed a significant reduction in glucose and insulin levels and HOMA-IR index. This reduction was associated with a significant change in serum lipid profile and liver enzyme levels (Samuel & Shulman, 2016). Our study has shown that resveratrol, metformin, or red grape extract can greatly improve insulin resistance (IR) and reverse changes in serum lipid profile and liver enzyme levels. High levels of dietary fat have been linked to the development of hyperglycemia and IR. While fat slightly affects serum insulin concentration and plasma glucose levels in the short term, prolonged exposure to high-fat diets can lead to lipogenesis, which results in a significant elevation in triglycerides and cholesterol in an insulin-independent manner. This leads to the accumulation of lipotoxic fat metabolites in skeletal muscle and impairs insulin signaling, causing IR primarily in the liver and muscles. IR and hyperinsulinemia are often associated with a shift in cholesterol metabolism, leading to increased synthesis and decreased absorption due to higher levels of triglycerides and free fatty acids (Hoenig & Sellke, 2010). High glucose concentration in the blood, along with insulin and value levels, may indicate the quality of the high-fat diet, which in turn helps to have insulin resistance. This resistance can be activated by the sebaceous secondary products that stimulate the C-JUN-N-Signing path in the insulin target cells. Moreover, excessive glucose levels can enter the basic synthesis path of glucosamine and other hexosamine derivatives, which may encourage glucose intolerance (Reda et al., 2022).

It is now known that insulin resistance is linked to food quality and the resulting diseases, especially fatty liver, as shown in Figure 2. The study begins to explain the effect of red grape extract on insulin resistance and the extent to which fatty liver improves when tested on experimental mice. HPLC results revealed the presence of resveratrol, which is one of the most important and prominent phenolic compounds in grape leaf extract. Attention was paid to studying its effect through molecular modeling against two important playmakers siturin-1 (SIRT-1) and phosphordiesterases (PDE).

The advantages of resveratrol have been associated with its molecular configuration, which enables it to interact with other molecules. Resveratrol consists of two aromatic rings connected by a styrene double bond. The number of hydroxyl groups present determines the differentiation of the aromatic ring. The distinction between cis-resveratrol and trans-resveratrol is based on the positioning of the molecule attached to the styrene double bond. Trans-resveratrol has two derivatives known as RSV1 and RSV2 (fig. 4) (Yanti, Chien, & Agrawal, 2022).

Glucose is the primary natural trigger for insulin release from pancreatic cells. The process of insulin secretion occurs in two stages Cyclic nucleotides (cAMP) and Cyclic guanosine monophosphate (cGMP), with each phase relying on boosting the metabolic pathway by around 50%. When blood glucose levels rise, there is a notable increase in glucose absorption and metabolism within pancreatic cells. This rise in the ATP/ADP ratio causes ATP-sensitive K+ channels to close, leading to membrane depolarization, the opening of voltage-gated Ca2+ channels, and the entry of calcium ions. The elevated concentration of intracellular calcium then prompts the release of insulin granules. Additionally, insulin secretion is also prompted by an increase in cAMP levels (Kilanowska & Ziółkowska, 2020).

Cyclic nucleotides, particularly cAMP, are crucial in regulating various physiological and pathophysiological processes. cAMP is involved in energy metabolism, triglyceride lipolysis, gluconeogenesis, glycogenolysis, and thermogenesis. cAMP enhances insulin secretion stimulation due to glucose and affects the performance of adenylyl cyclases isoforms. A strong interdependence between cAMP and Ca2+ affects the performance

of adenylyl cyclases. cAMP stimulates exocytosis of insulin in a PKA-independent manner. cAMP increases the efficiency of cAMP receptors like PKA and generates tissue-specific PDE activity. An increase in cAMP leads to the dissociation of PKA active catalytic subunits and phosphorylation of various proteins affecting metabolism, proliferation, and insulin secretion. cAMP plays a key role in regulating insulin secretion, with basal cAMP levels necessary for glucose-induced insulin secretion (Tian et al., 2012). cGMP plays a role in insulin secretion and glucose metabolism, regulating glucose metabolism through NO activation. β cells in the pancreas induce apoptosis and potentiate insulin secretion, but their course remains unclear. Controlling cGMP concentration may lead to new diabetes treatment directions (Undank et al., 2017). Numerous hepatic diseases are becoming more familiar globally and are thought to be serious health issues. One of the primary strategic regulators of several biological processes, such as the cell cycle, apoptosis, mitochondrial biogenesis, insulin secretion, redox balance, inflammation, and inflammatory responses, are sirtuins. The most well-known and extensively researched sirtuin associated with longevity and health status is SIRT1. SIRT1 is a class III (NAD+-dependent) histone deacetylase (HDAC) that mimics most metabolic responses to calorie restriction. Consequently, it could make sense to use SIRT1 signaling pathway targeting as a therapeutic strategy to treat a variety of illnesses, including metabolic disorders (Sayed, Hassanein, Salem, Hussein, & Mahmoud, 2020).

Profiling of the binding site position and binding residue in 3D, a molecular docking study was conducted against siturin-1 (SIRT-1) and phosphordiesterases (PDE). Through the use of distinct SIRT1 and PDE proteins crystal structures, our computational method was able to identify potential resveratrol binding sites to several pockets both inside and outside the catalytic domain of the enzyme. Resveratrol bind with a higher affinity to SIRT1 by made three hydrogen bonds, this result was matched with previous bioinformatics analysis revealed that RSV bound all SIRT1 complexes with a higher affinity (Livraghi et al., 2024). The present study's results suggest that resveratrol ameliorates insulin resistance through SIRT1 expression that occurs in adipose tissues. Several mechanisms explained how resveratrol can control insulin resistance; one of them is the activation of SIRT1 by resveratrol, which increases AMP-activated protein kinase (AMPK) activity, which in turn controls diabetes (Figure 3). AMPK is expressed in various tissues, especially skeletal muscle and adipocytes. Skeletal muscle is a major site for glucose uptake and glycogen synthesis (~80%). The postprandial elevation of blood glucose triggers insulin release, which suppresses production of glycogen in muscle cells and activates glucose transporter type 4 (GLUT4) transporters (highly expressed in muscles) to facilitate uptake of glucose into muscle cells for glycogen synthesis, thereby reducing blood glucose concentration. Also, the activation of AMPK up-regulates mitochondrial biogenesis, inhibiting triglyceride synthesis and stimulating glucose uptake and fatty acid oxidation in the skeletal cells, improving insulin sensitivity (Yanti et al., 2022).

Our computational data suggests that resveratrol bind with a higher affinity to PDE. Resveratrol molecule was made four hydrogen bonds with it. Resveratrol activates PDE, which regulates the intracellular cAMP levels that suppress adiponectin secretion and expression that control insulin sensitivity. The phosphodiesterase family (PDEs) comprises 11 structurally similar isoforms. These enzymes have various applications in diabetes, and their versatility is continually surprising. Enhancing our understanding of phosphodiesterases will aid in effectively targeting them and minimizing side effects to fully utilize their potential as therapeutic targets in diabetes. It is suggested that increasing intracellular cAMP and Ca2+ ions boosts the activity of PDE. These enzymes have dual specificity for breaking down both cAMP and cGMP, albeit with different affinities. Researches indicate that PDE may have a significant role in pancreatic β cells concerning Ca2+-dependent signaling pathways, which are closely linked to insulin secretion (Kilanowska & Ziółkowska, 2020). Elevating of cGMP levels through the inhibition of PDE, especially PDE 5, may serve as a preventative strategy in mitigating the onset of insulin resistance by enhancing endothelial function. The sustained activation of the nitric oxide (NO)/cGMP/protein kinase G (PKG) pathway through PDE-inhibitors as resveratrol may promote increased blood circulation and enhance insulin responsiveness in facilitating muscle glucose uptake, consequently leading to a reduction in plasma glucose concentrations.

Based on the findings of previous researches in this area as well as the current molecular modeling, the mechanism of action of resveratrol and its effects on metabolism have been clarified. According to previous research, resveratrol raises cAMP levels by competitively inhibiting the PDEs that break down cAMP. Resveratrol's metabolic effects depend on the activation of AMPK, which is triggered by cAMP-dependent pathways. The metabolic advantages of resveratrol, such as defense against diet-induced obesity and enhanced mitochondrial function, physical survival, and glucose tolerance in mice, are brought about by PDE inhibition (Chung, 2012). Considering the aforementioned, resveratrol is regarded as a significant PDE inhibitor that may help treat metabolic disorders linked to obesity and fatty liver.

In summary, Resveratrol can directly interact with and stimulate sirtuins, which act as cellular detectors for NAD+. A key aspect of its functionality involves modulation of SIRT1 and AMPK in a dose-dependent and reciprocal manner. When present in lower concentrations, resveratrol induces SIRT1 to deacetylate and activate liver kinase B1 (LKB1), an upstream kinase of AMPK. This process increases AMPK activity, which triggers energy breakdown

and raises cellular NAD+ levels. Conversely, resveratrol can potentially hinder mitochondrial ATP production at higher concentrations, thereby enhancing the cellular AMP-to-ATP ratio. As a result, AMPK is stimulated, leading to autophagy induction and fresh mitochondria production. Concurrently, SIRT1 inhibits NF-κB, thereby eliciting anti-inflammatory and hepatoprotective outcomes that contribute to the mitigation of liver injury and tissue necrosis. This cascade of events also diminishes the concentrations of AST, ALT, and ALP in the circulatory system, thereby influencing insulin resistance. The regulatory effects of resveratrol on SIRT1 and AMPK play a role in its potential properties.

Moreover, resveratrol can stimulate AMPK through mechanisms involving cAMP and calcium, which benefit aging and cellular senescence. By inhibiting PDE, resveratrol elevates the levels of cAMP and Ca2+ within cells, thereby activating the AMPK pathway. This pathway enhances autophagy and boosts NAD+ levels, subsequently enhancing SIRT1 activity that encourages the expression of antioxidant genes. Moreover, due to resveratrol's interference with PDE function, cAMP levels rise, which triggers the activation of SIRT1, which in turn leads to an increase in phosphorylation processes, then SIRT1 activation ultimately reduces the production of hepatic enzymes like AST, ALT, and ALP and reduces inflammation due to the reduction of insulin resistance (fig. 5).

sirtuin 1, phosphodiesterases (PDE), AMP-activated protein kinase–human sirtuin 1 (AMPK), glucose transporter type-4 (GLUT4), Guanosine 3',5'-cyclic monophosphate (cGMP)

4. Conclusion

In conclusion, our investigation aimed to evaluate the efficacy of red grape leaf extract in addressing the biochemical changes associated with NAFLD. The phenolic compounds in grape leaf extract, especially resveratrol, demonstrated synergistic effects on insulin resistance and liver biochemical parameters. Compared to control groups, recent in vivo experiments have shown significant improvements in various biochemical markers, including serum lipid levels, liver enzyme activity, and HOMA-IR. Furthermore, molecular docking analyses revealed potential interactions between resveratrol and SITR 1 and PDE proteins, suggesting its involvement in mitigating hepatic steatosis through autophagy promotion via these signaling pathways. These findings offer valuable insights into the role of resveratrol in addressing insulin resistance within the context of NAFLD treatment.

Future research endeavors will explore different formulations of grape leaf extract as functional food products to determine the most effective means of harnessing its therapeutic benefits for individuals with NAFLD. This avenue of exploration holds promise for developing innovative strategies to combat this prevalent metabolic disorder.

Author contributions

Naglaa M. Hamdy, and Haman M. El-Tantawy performed all aspects of this work.

Acknowledgment

None declared.

Competing financial interests

The authors have no conflict of interest.

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