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Anti-Obesity Efficacy of Lipoidal Matter of *Vitis Vinifera* Leaves in Rats

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Abstract

Background: Obesity is one of the most prevailing health issues globally. *Aims*: The current study was conducted to evaluate the biochemical effects of *Vitis vinifera* leaves petroleum ether extract (VLPE) against high-fat diet induced obesity in rats. *Methods*: VLPE was administrated in a dose 200 mg/kg body weight. Liver enzymes, lipid profile, oxidative stress, apoptotic and inflammatory biomarkers as apelin, paraxonase -1 (PON-1), Nuclear Factor Kappa beta (NFkB), Monocyte chemoattractant protein-1 (MCP 1) and (B-cell lymphoma 2) BCl2 were assayed. Also, glucose, leptin and lipase levels were evaluated. Moreover, the phytochemicals profile of VLPE was characterized through chromatographic and spectroscopic tools after saponification. *Results:* Post-treatment of obese rats with VLPE**,** a significant decrease in the bodyweight gain was detected compared control group. In addition, reduction in MDA while an elevation in GSH, apelin and PON1 levels post treatment of obese -rats with VLPE was observed. Also, a noticeable improvement in other parameters was recorded upon treating HFD-rats with VLPE. Post treatment of obese rats with *Vitis vinifera*; normal histological structure of hepatic lobule with mild inflammatory cells infiltration in the portal area was examined. Kidney of obese rats showed vacuolar degeneration in the epithelial cells lining the tubules, while obese rats treated with *Vitis vinifera e*xtract and Orlistat drug showed no vascular congestion and no inflammation. Concerning the characterization of VLPE, twenty- three compounds, as well as fifteen fatty acids, were identified in unsaponifiable and saponifiable fractions, respectively. Unsaponifiable fraction constituted of hydrocarbons (13.86% of total unsaponifiable matter), fatty alcohol (7.20%) and sterol (2.65 %) and triterpene compounds (2.09%). Column chromatography of the USM afforded lupeol, cholesterol, campesterol and β-sitosterol in addition one fatty alcohol 1-triacontanol. *Conclusion*: According to the finding results, a lipoidal matter of *Vitis vinifera* leaves possesses multi-functional anti-obesity activities that can be used as a phytochemical functional food.

Key words: Vitis vinifera, sterol, triterpene, hypocholesterolemic, anti-oxidative stress, lipase , apelin , leptin; apoptosis, anti-inflammation

1. Introduction

Obesity is one of the most prevailing health issues global disease caused by the interaction of a myriad of genetic, dietary, lifestyle, and environmental factors **[1].** Obesity often associated with metabolic disorders (diabetes and hypertension) and cardiovascular diseases as well as chronic diseases including stroke, osteoarthritis, some cancers and inflammation-based pathologies. Lowering of obesity and its complication incidence is still a globally challenging issue.

Currently, anti-obesity drugs available in the market have reverse effects including increased blood pressure, headache, dry mouth, insomnia, and constipation where their mechanism of action through inhibition of pancreatic lipase (Orlistat) or anorectic or appetite suppressant (subutramine) **[2,3] .** Recently many researchers focus on natural products to treat, reduce, or prevent obesity regarding their safety availability and its minimal costs. The efficacy of natural products to counteract obesity through regulation of various pathways, including lipid

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absorption, energy intake and expenditure, increasing lipolysis, and decreasing lipogenesis, differentiation and proliferation of preadipocyte was investigated by many researchers **[4-7] ,** reviewed the anti-obesity effects of numerous potential extracts and their active ingredients derived from natural sources such as plants (fruits, vegetables, grains, and herbs) have been investigated.

Grapevine (*Vitis vinifera* L.) leaves are considered one of a disregarded agricultural by-product which can be an endless source of bioactive phytochemicals. They have been consumed as food in Middle East nations and have found applications in folk medicine **[8].**

A majority of the studies on the polyphenolic composition of Vitis leaves have been reported including their different biological effects. The bioactive phyto-constituents such as, phenolic, flavonoids, tannins possessed strong antioxidant, antidiabetic, antiinflammatory, hepato-protective, anti-hypercholesterolemic and anti- Alzheimer effects **[9-13].** In contrast, little information on the grapevine leaves lipophilic composition as well as its bioactivity was noted. Nevertheless, several authors have reported the lipophilic contents of cuticular waxes focusing triterpenes and sterols compositions in different varieties of Vitis spp. leaves **[14-17].**

Batovska and co-authors [14, 15], reported that the leaf surface components of four Bulgarian *Vitis vinifera* L. seedlings constituting mainly triterpenoids, sterols, fatty acids, esters and heterocyclic compounds. Moreover, the qualification and quantification of triterpene and sterol contents in leaves of eight grapevine species revealed variation in cholesterol, campesterol, stigmasterol, clionasterol and sitosterol in addition to pentacyclic triterpenoids of ursane-, oleanane-, lupane- and taraxerane-type skeletons content depending on the cultivar and variety of grapevine**[18].**

Several preclinical studies paid attention to phyto extracts as anti-obesity with the various mechanism of action contributed to bioactive metabolites diversity. So, the current study was designed to evaluate the anti-obesity potential of *Vitis vinifera* leaves lipoidal matter, and to qualify its phytoconstituent. Further, positive results can promote uses of grape leaves as afunctional food.

2. Materials And Methods

2.1. Phytochemical study

2.2. General procedure

GC/MS investigation of the lipoidal substance was received utilizing a Thermo Logical (Waltham, Mama), Follow GC Ultra and ISQ120602 ISQ Single Quadrupole MS, TG-5MS melded silica slender section (30 m x 0.25mm x 0.1mm film thickness). For GC/MS discovery, an electron ionization framework with ionization energy of 70 eV was utilized. The inactive gas helium was utilized as a transporter gas, at a stream pace of 1 ml/min. The injector and MS move line were set at 280 ℃. Temperature was modified for unsaponifiable matter (USM) examination at a temperature 50 ℃ (2 min), 50–150 ℃ at a pace of 7 ℃/min, 150–270 ℃ at a rate 5 ℃/min, 270–310 ℃ as a last temperature at an expanding pace of 3.5 ℃/min, run time(min): 59.72. While, temperature customized of unsaturated fat methyl esters (Popularity) was 150–280 ℃ at a pace of 5 ℃/min. NMR estimations were done utilizing Jeol EX-500 spectroscopy; 500 MHz (1H NMR) and utilizing CDCl3 as dissolvable. Mass spectrometric investigations were performed on a Finnigan Tangle 112, electron sway ionization at 70 eV.

2.3. Plant Material

Leaves of red grape (*Vitis vinifera* L.) cv. Flame Seedless, belonging to the family *Vitaceae*, were collected during lush vegetation period in May 2018 from Elzomor Farm, Alexandria road. Botanical identification, by Prof. Aisha Saleh Abd elrahman department of Viticulture Research ,Horticulture Research Center, Ministry of Agriculture, Giza, Egypt. A voucher specimen is deposited under the number A 09 at Pharmacognosy Department, National Research Centre. The leaf petioles will be carefully manually separated and leaves were dried at room temperature under shaded condition, and then coarsely powdered and will be stored in polyethylene plastic bags in a dry place.

2.4. Extraction of lipoidal matter

The dried powdered of *Vitis vinifera* L. leaves (1kg) was submitted to extraction at room temperature with petroleum ether (40-60 ℃) at a solid to liquid ratio of 1:10 (w/v). The extract was evaporated under vacuum in rotary-evaporator at 37 ℃ yielded a semi solid material (4.5 g).

2.5. Saponification of petroleum ether extract

The isolation of unsaponifiable matter (USM) and fatty acids from petroleum ether extract as well as preparation of methyl ester of fatty acids (FAME) were prepared according to the method described by Matloub et al. [19]. The USM and FAME were exposed to gas chromatography/mass spectrometry (GC/MS) investigation. The distinguishing proof of the mixtures was refined by looking at their maintenance times and mass information with those of the library (Wiley Int.USA) and NIST (Nat. Inst. St. Technol., USA) and/or The Lipid Web.

2.6. Colum chromatography

The unsaponifiable part was chromatographed on silica gel utilizing VLP and eluted with a combination of solvents including n-hexane, dichloromethane and methanol with expanding extremity. Parts of 100 ml were gathered and those with comparable attention profiles, were joined together to afford sixteen subfractions. For detecting terpenoids, the subfractions were spotting on TLC Silica gel 60 F_{254}, using developing system benzene: ethyl acetate (8:2 v/v) and sulfuric acid reagent (20% in ethanol). Subfractions; A (80% hexane/ dichloromethane), B (100% dichloromethane), C (80% dichloromethane/ methanol) and E (50% dichloromethane/ methanol) showed promising 7 spots which gave either purple, reddish or violet colour when sprayed with 20% sulfuric acid. After purification, 1-5 compounds responded positively to the Liebermann-Buchard were obtained.

Compound 1: Colorless crystals, isolated from subfraction B, R_f: 0.63 (benzene: ethyl acetate- 8: 2 v/v). m. p. 212-214 °C. MS (70 eV), *m/z* (rel. int) 426 $[M]^+$which corresponds to the molecular formula ($C_{30}H_{50}O$) (21), 411 (13), 408 (10), 393 (5), 364 (15), 218 (62), 207 (95), 203 (62), 189 (6), 139 (7),125 (18), 57 (100) and 55 (94). ^{1}H-NMR ($CDCl_3$, 400 MHz), δ 0.78(3H, s, Me-28), 0.80 (3H, s, Me-23), 0.89 (3H, s, Me-24), 1.05 (3H, s, Me-27), 0.96 (3H, s, Me-25), 0.98 (3H, s, Me-26), 1.69 (3H, s, Me-30), 2.40 (1H, m, H-19), 3.65 (1H, dd, *J* = 11.2, 5.2 Hz, H-3), 4.70 (1H, br s, H_a-29), 4.58 (1H, br s, H^b-29), 1.58, 1.27, 2.15, 2.16

Compound 2: White needles, m. p. 139-141 °C, isolated from subfraction B, R_f: 0.48 (benzene:ethyl acetate- 8: 2 v/v). MS (70 eV), *m/z* (rel. int) 414 $[M]^+$ corresponding to $C_{29}H_{50}O$, 399, 396, 381, 367, 329, 303, 273, 255, 231, 213,107, 81, 69 and 55. ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 0.68 (3H, S, Me-18), 0.81 (3H, d, *J* = 6.4 Hz, Me-26), 0.83 (3H, d, *J* = 7.4 Hz,Me-27), 0.84 (3H, d, *J* = 7.4 Hz, Me-29), 0.93 (3H, d, *J* = 6.6 Hz, Me-21), 1.01 (3H, s, Me-19), 3.52 (1H, m, H-3), 5.35 (1H, d, *J*=4.8, H-6)

Compound 3: White amorphous powder, m. p. 160-162 °C, isolated from sub-fraction C, R_f: 0.28 (benzene: ethyl acetate 8: 2 v/v). 400 $[M]^+$ corresponding to the molecular formula $C_{28}H_{48}O$, 385, 382, 367, 315, 289, 273, 261, 255, 231, 213, 145,105, 81, 67, 55 and 43. ^{1}H-NMR: (500 MHz, $CDCl_3$): δ 0.68 (3H, S, Me-18), 0.84 (3H, d, *J* = 6.8 Hz, Me-26), 0.80 (3H, d, *J* = 7.2 Hz, Me-27), 0.79 (3H, Me-28), 0.92 (3H, d, *J* = 6.6 Hz, Me-21),1.01 (3H, s, Me-19), 3.51 (1H, m, H-3), 5.34 (1H,br s, H-6).

Compound 4: Colourless needles, m. p. 148-149 °C, isolated from subfraction E, R_f: 0.27 (benzene: ethyl acetate 8: 2 v/v), MS (70 eV), *m/z* 386 $[M^+]$ corresponding to ($C_{27}H_{46}O$), 371, 368, 353, 301, 273, 255, 247, 231, 213. ^{1}H-NMR: (400 MHz, $CDCl_3$) : δ 0.68 (3H, S, Me-18), 0.85 (3H, d, *J* = 6.2 Hz, Me-26, 27), 0.91 (3H, d, *J* = 6.6 Hz, Me-21),1.01 (3H, s, Me-19), 3.52 (1H, m, H-3), 5.35 (1H, d, *J*=4.8, H-6).

Compound 5: Amorphous powder, isolated from sub-fraction A, R_f: 0.70 (benzene: ethyl acetate- 95: 5 v/v), m. p. 87 ° C. MS (70 eV) showed *m/z* 438 $[M]^+$corresponding to the molecular formula $C_{30}H_{62}O$ with base peak fragment at *m/z* 97 and other principle fragments 420, 392, 364, 351, 339, 325, 292, 283, 264, 255, 222, 111, 83, 69, 57. ^{1}H-NMR ($CDCl_3$, 400 MHz) showed chemical shifts at δ 0.88 (t, *J* = 8.0 Hz, 3H, <u>CH₃</u>), 1.25 (54H, broad S, <u>CH₂</u> H3–H29), 1.57 (<u>CH₂</u>), 3.64 (t, *J* = 6.8 Hz, CH_2O<u>H</u>).

Palmitic acid: Obtained as a white crystalline precipitated, by shaking the methanol soluble fraction from petroleum ether extract with ethyl acetate. R_f: 0.70 (benzene: ethyl acetate- 95: 5 v/v), m. p. 63-64 ° C. MS (70 eV) showed *m/z* 256 $[M]^+$ corresponding to the molecular formula $C_{16}H_{32}O_2$with base peak fragment at *m/z* 73 and other principle fragments 227, 213, 199, 185, 171, 157, 143, 129, 97, 85, 59, 57. ^{1}H-NMR (DMSO, 500 MHz) showed chemical shifts at δ 0.83 (d, *J* = 5.0 Hz, 3H, <u>CH₃</u>), 1.23 (22H, broad S, -(<u>CH₂</u>)$_{4\text{-}14}$-), 1.48 (-C<u>H</u>$_2$CH$_2$COOH), 2.50 (s, -C<u>H</u>$_2$COOH). ^{13}C-NMR (DMSO, 125 MHz) showed

signals at δ 174.50 (C1), 33.68 (C2), 24.51(C3), 28.57(C4), 28.76 (C5), 28.93 (C6), 29.04(C7), 29.04 (C8), 29.05 (C9), 29.05 (C10), 29.05 (C11), 31.32 (C12), 29.05 (C13), 29.05 (C14), 22.12 (C15), 13.96 (C16).

2.7. Biological activity

2.7.1. Chemicals and Reagents

All chemicals of analytical grade produced from El Nasr Chemical Company, Cairo Egypt, Fluka Sigma Chemical Company, NY, USA .Colorimetric diagnostic kits and ELIZA kits were purchased from Biodiagnostic Chemical Company, Cairo, Egypt, and Sigma Chemical Company, NY, USA. Orlistat was purchased from local Pharmacy, Cairo, Egypt.

2.7.2. Animals

Male albino rats (n=50) weighted (150±20 g), were acquired from the Creature Place of the Public Exploration Community (NRC). Creatures were isolated and permitted to adjust for 10 days prior to starting experimentation. They were housed 10 for each pen under temperature controlled climate (26-29°C) with a fixed light/dim cycle with free admittance to water and food. All methodology of the current examination were performed by the Medical Division of NRC, Egypt, given that the creatures won't endure at any phase of the analysis. The ethical approval no: 4443042021.

2.7.3. Acute toxicity

Treatment of rats with dried powdered of *Vitis vinifera* L. leaves will be carried out to determine the oral LD50, *Vitis vinifera* L. leaves biomass suspension in water solution was administered to Waster rats (4/sex/group) as a single oral dose of 50-5000 mg /kg body weight via gavage. Animals were observed for 24 h for signs of morbidity or mortality. The control group was treated with water vehicle. The animals were observed during 24 h investigation.

2.7.4. Induction of obesity in rodents

Obesity was prompted in rodents as indicated by the technique for Adaramoye et al.[20], by taking care of rodents high-fat eating routine (cholesterol), cholesterol was orally administrated at a dose (30 mg/0.3 ml olive oil/kg creature) seven times each week for twelve continuous weeks, fat was blended in with ordinary eating regimen (one kilogram of creature fat was added to 5 Kgs of typical eating routine), the event of obesity was dictated by estimating body weight acquire rates.

2.7.5. Doses and routs of administration

Obese rats received an oral dose of the anti-obestic orlistat as reference drug was orally administrated at a dose 12 mg/kg b.wt. . The drug was dissolved in distilled water for oral administration for 7 consecutive weeks to obese-induced rats **[20]** . *Vitis vinifera* petroleum extract was administered orally for 12 weeks in a dose 200 mg/kg body weight by oral gavage (1/25 LD50)**.**

2.7.6. Experimental design

Fifty male Wistar albino rats (5 to 6 weeks old) weighing at 150 .00 ± 20 g (mean ± SD) (weight of rats on the day received from supplier) after adaptation period to the environment, the rats were randomly divided into five groups (n= 10/ group) as follows:

Group (1): Normal Diet (ND) control rats.

Group (2): Normal Diet rats and treated with 200 mg/kg body weight of VLPE for 12 consecutive weeks.

Group (3): High Fat Diet (HFD) treated (30 mg/ 0.3 ml olive oil /kg animal) seven times a week for twelve consecutive weeks.

Group (4): obese rats treated for 12 weeks with 200 mg /kg body weight of VLPE

Group (5): obese rats treated for 12 weeks with anti- obestic drug orlistat as standard drug 12 mg/kg body weight for 6 consecutive weeks. Medical issue of all rodents were checked day by day and no antagonistic occasions were noticed all through the investigation. Toward the start of the examinations the weights of all rodents were recorded at 155.00 ± 5.00 g (mean ± SD) (weight of rodents following 10 days of acclimatization). All investigations and biochemical examination were directed utilizing 50 rodents with three-fold estimations.

2.7.7. Biochemical analysis

Blood glucose level was determined [21], using colorimetric kits. Determination of leptin [22] and pancreatic lipase enzyme [23] were carried out using ELIZA kits. Lipid profile (TC, TG, HDL and LDL) was determined according to Richmond [24], Fassati and Prencipe[25] and Burstein[26], respectively, using colorimetric diagnostic kits. Also, liver marker enzymes were estimated in serum using colorimetric diagnostic kits, where aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [27],

alkaline phosphatase (ALP) [28] (28) and gama glutamyl transferase (GGT)[29] levels were measured. Inflammatory and apoptotic markers were carried out in serum using Eliza kits. BCl2 [30], MCP1 [31],NFkB [32], apelin [33] and PON [34] were determined. GSH was tested in liver homogenate as indicated by Moron et al. [35] The he strategy depends on the advancement of a generally steady yellow shading when 5, 5'- dithiobis-2-nitrobenzoic corrosive (DTNB) is added to sulfhydryl compounds. Malondialdehyde (MDA) was tested in liver tissue depend on Buege et al. [36] method. MDA is precarious compound that disintegrated to frame an unpredictable arrangement of receptive carbonyl mixtures. Polyunsaturated unsaturated fat peroxides produced malondialdehyde which has been utilized as an indicator of lipid peroxidation measure.

2.8. Histopathological analysis

Slices of kidney and blood vessels were fixed in 10% formaldehyde and implanted in paraffin. 5 mm thickness sections were stained with hematoxylin and eosin (H&E), and then examined under a light microscope for the pathological alterations **[37].**

1.9. **Statistical analysis**

All data were expressed as mean ± S.D. for each group. One-way analysis of variance (ANOVA), SPSS version 8, combined with Co-stat Software Computer Program, was used, where different letters are significant at P≤0.05.

3. Results

Vitis vinifera L. cv. Flame Seedless leaves (VLPE) yielded 4.57% w/w of petroleum ether extract. Saponification of VLPE afforded 67.80 % of unsaponifiable matter (USM) and 30.36 % of the fatty acids. GC/MS analysis of USM allowed to identification of 23 compounds accounting for 75.49% of total unsaponifiable matter represented as Hydrocarbons (13.86%), fatty alcohol (7.20%), one monoterpene (1.99%), one sesquiterpene (1.30%), two diterpenes (17.53%), sterols (3.19%) and one triterpene (2.09%). Phytols (17.53%) and 6, 10, 14-trimethyl-2-pentadecanone (16.36%) are predominant unsaponifiable matters of VLPE. Fatty alcohol; E-2-tetradecen-1-ol, 1-octacosanol and 1-hexacosanol were identified in leaves of *Vitis vinifera* L. cv. Flame Seedless for first time (Table 1).

In previous study, the total content of sterols ranged from (0.6 to 1.7 mg/g) of dry leaf weight and β-sitosterol was the most abundant phytosterol of all tested grapevines leaves **[18].** The sesquiterpene E-nerolidol and monoterpene α-pinene were only detected in our sample while other sesquiterpene; α-farnesene and Trans α-bergamotene as well as other monoterpenes; terpinolene and carene were identified alongside E-nerolidol and α-pinene in other cultivars of grapevine leaf tissue **[38].** Moreover, GC/MS analysis of fatty acids methyl esters led to identify fifteen fatty acids (Table 2). The saturated fatty acids are the main constituents of VLPE account 82.07% of total identified fatty acids. In agreement with study of **Salvador et al. [39],** palmitic acid (64.14%) represented as predominant fatty acid as well as other saturated fatty acids behenic (C22:0), tricosanoic (C23:0), lignoceric (C24:0) and cerotic acid (C26:0) were detected in traces in VLPE were previously identified in *Vitis vinifera* leaves.

In contrast, previous study reported linoleic acid (ω-6) as the dominant in various variety of *Vitis vinifera* L. **[40, 41]** .While, the two essential fatty acids linoleic (5.34%) and linolenic acids (11.49 %) were detected in a ratio ω-6/ω-3 (1:2) in VLPE. Lower content of unsaturated fatty acids particularly the α-linolenic (C18:3) fatty acid in our sample may be due to picking the healthy leaves before exposure to chemical additive and microbial diseases aiming to avoid stress. Different study proved that high content of unsaturated fatty acids is important for resistant grapevine genotypes particularly the α-linolenic (C18:3) fatty acid that had been associated to resistance against fungal and bacterial pathogens **[42, 43]**. Moreover, the study of **Batovska et al. [14]** proved that significant variations in the metabolite composition including sterols, terpenes, fatty acids and heterocyclic compounds of the leaf surface layers depending on the environmental factors or to the plant development.

GC/MS analysis of unsaponifiable matter didn't reveal triterpenes compounds that led us to fractionate unsaponifiable fraction over column chromatography of silica gel. Column chromatography of the USM afforded one triterpene lupeol, three sterols cholesterol, campesterol and β-sitosterol in addition one fatty alcohol 1-triacontanol. The ^{1}H-NMR spectrum of compound 1 displayed seven methyl singlet signals resonating at δ 0.78, 0.80, 0.89, 0.96, 0.98, 1.05 and 1.69. In addition, two broad singlet signals at δ 4.58 and 4.70 for exocyclic olefinic protons of C-29 together with characteristic fragments at m/z 411, 408, 393, 364, 218, 207, 203, 189 suggested that the compound 3 possesses a

lupeol-type triterpenoid. By comparing aforementioned data of **compound 1** is closely agreement to that reported for lupeol **[44].** It was previously reported from *Vitis vinifera* leaves tissue and leaf cuticular waxes **[17, 38].**

The H NMR spectra of compounds 2, 3 and 4 displayed two signals with high chemical shifts values for olefinic proton and proton connected to C-3 hydroxyl group which resonated as broad doublet at δ 5.35 with J = 4.8 Hz and other resonated as a multiplet at δ 3.52, respectively as well as two tertiary methyl groups resonated as singlet signal at δ 0.68 and 1.01 for methyl protons of 18 and 19, respectively which were characteristic for phytosterols. Also, mass spectrum of compounds 2, 3 and 4 showed molecular weight at m/z 414 $[M]^+$, 400 $[M]^+$ and 386 $[M]^+$, respectively with fragmentations characteristic for *β*-sitosterol, campesterol and cholesterol agreed with the data of Matloub et al. [44], Hashem et al. [45] , Suttiarporn et al. [46]. The H NMR data of compound 5 displayed a triplet signal at δ3.64 for an α-hydrogen adjacent to a hydroxyl group, indicating a CH_2OH group, and a triplet signal at δ0.88 for a terminal CH_3 group as well as a broad signal integrated for 54H at δ1.25 and a multiplet signal with 2H at δ1.57 indicated the presence of $[CH_2]_{27}$ and CH_2, respectively. In addition, mass spectral data of compound 5 showed typical alcoholic hydrocarbon pattern for an aliphatic straight chain primary alcohol, giving molecular weight at *m/z* 438 $[M]^+$ corresponding to 1-triacontanol. The mass spectra, physical and NMR spectral data concur with those reported by Mori et al. [47]. Long-chain aliphatic alcohols, 1-triacontanol as well as hexacosanol and octacosanol were detected previously in *Vitis vinifera* leaves as the major components [39].

Table 1 GC/MS analysis of unsaponifiable matter isolated from *Vitis vinifera* leaves

Compounds	Rt	%	BP m/z	Mwt m/z	Main fragments (m/z)
2-Methylenebicyclo[2.2.1]-heptane	13.54	1.61	93	108	53, 67, 77, 79, 81, 91
3-hydroxy-2-methyl-2-Cyclopenten-1-one	15.96	1.72	112	112	41, 55, 56, 69, 83, 97
(-)-β-Pinene	18.27	1.99	93	136	41, 53, 69, 77, 79,107
Butylated hydroxytoluene	19.12	3.84	205	220	41, 57, 67, 81,91,105, 145, 177, 206
Dihydroactinidiolide	20.37	3.18	111	180	109, 137, 67, 55, 124, 152,165
E-2-tetradecen-1-ol	27.02	3.01	57	212	41, 68, 82, 96, 109, 124, 137,194
6,10, 14-Trimethyl-2-pentadecanone	27. 29	16.36	43	268	58, 71, 85, 95, 109, 124, 137, 210, 235, 250
✓ 2-methyl-7-Octadecyne	27.59	1.34	81	264	67, 82, 95, 109,123, 138, 179, 249
✓ 9-Eicosyne	28.01	2.51	67	278	43, 57,55, 81, 95, 109, 123, 208, 236
✓ E-Nerolidol	28.89	1.30	69	222	41, 43, 55, 81, 93, 107, 121, 136, 161, 148, 179, 189,204
✓ Isophytol	29.50	1.73	71	296	57, 82, 97, 111, 123, 141, 281
✓ Phytol	32.91	15.80	71	296	57, 82, 95, 109, 123,140, 278
✓ n-Tetracosane	36.26	1.86	57	324	43, 71,85,99,113,127,141
✓ 4,8,12,16-Tetramethylheptadecane-4-olide	37.43	2.18	99	324	58, 57, 69, 83, 114, 126
✓ 1-Hexacosanol	38.30	2.01	55	382	43, 69, 83, 97, 111, 364
✓ Pentacosane	39.67	2.16	43	352	71, 85, 99, 267, 295, 323
✓ Octacosanol	42.85	2.18	57	410	55, 69, 83, 97, 111
✓ Squalene	44.97	1.61	69	410	55, 81, 95, 109, 121,123, 137
✓ nonacosane	46.51	2.56	57	408	71, 85, 99, 113, 365, 379
✓ β-Sitosterol	48.85	1.26	414	414	213,273,303,329, 355,396,381
✓ n -Hentriacontane	50.21	1.80	57	436	43,71,85,99,113
Stigmast-4en-3-one	55.22	1.39		412	397, 271, 135, 147, 96
Betulin	61.05	2.09		442	424, 411, 399, 393, 302, 207, 189, 107, 81,69
Total identified		75.49			
Hydrocarbon		13.86			
Fatty alcohol		7.20			
monoterpene		1.99			
sesquiterpene		1.30			
diterpene		17.53			
triterpene		2.09			
sterols		2.65			

Table 2: GC/MS analysis of fatty acids methyl esters isolated from *Vitis vinifera* leaves

Compounds	Rt	Relative %	BP m/z	M. wt. m/z	Main fragments (m/z)
Decanoic acid, methyl ester **Methyl caprate**	6.86	0.15	74	186	87,155,143,129,101
Tetradecanoic acid methyl ester **Methyl myristylate**	11.22	0.54	74	242	43, 55,57 ,87, 143,199, 213
12-methyltridecanoic acid methyl ester(iso} **Methyl isomyristate**	11.28	0.35	74	242	87, 59, 69,171,185,199, 211, 228
Hexadecanoic acid methyl ester	16.23	64.14	74	270	43,55,69,87, 143,129,227,241
Heptadecanoic acid methyl ester **Methyl margarate**	18.49	0.63	74	284	43.55, 87,143,129,157,185,241, 255
9,12-octadecadienoic acid methyl ester C18:2 **ω-6** **Methyl linoleate**	20.11	5.34	67	294	41,55, 81, 95,109,123,150,263
9,12,15-octadecatrienoate methyl ester C18:3 **ω-3** **Methyl linolenate**	20.29	11.49	79	292	67,95, 41, 108, 55, 236, 263
Octadecanoic acid methyl ester C18:0 **Methyl stearate**	20.82	6.78	74	298	87,43,57,143,199,255, 267
Eicosanoic acid methyl ester **Methyl arachidate**	25.14	2.14	74	326	87,43,55,143,199,227,295
14- oxononadec-10-enoic acid methyl ester	25.69	1.10	99	324	71,43,55,81,109,151, 194, 221,250, 293
Heneicosanoic acid methyl ester	27.17	0.47	340	340	74,87,143,241,283,297, 309
Docosanoic acid methyl ester **Methyl behenate**	29.16	3.63	354	354	74,87,143,255,283,311,323
Tricosanoic acid methyl ester	31.06	0.50	368	368	43, 55,57,74, 87, 129,143,325,337
Tetracosanoic acid methyl ester	32.90	1.98	382	382	43,57,74, 129, 143,283,339, 368
Hexacosanoic acid methyl ester **Cerotic acid methyl ester**	36.40	0.76	410	410	43, 57, 74, 87, 129, 143,199, 311, 367, 381
Total unsaturated fatty acid		17.93			
Total saturated fatty acid		82.07			

Concerning to palmitic acid, part of the dried petroleum ether extract was shaked with methanol, filtered, the filtrate was concentrated, shaked with ethylacetate and then water was added till slightly turbid, a white precipitate was obtained from the alcohol soluble fraction which was separated by filtration. H and C NMR as well as mass spectrum and melting point confirmed that the white precipitate was palmitic acid. Palmitic acid was found as predominant fatty acid petroleum ether extract. The mass spectra, physical and NMR spectral data are in agreement with those reported in the literatures of Di Pietro et al. [48].

3.1. Effects of VLPE on percent body weight gain and food intake in obese rats induced by HFD

Table 3 shows the effect of high fat diet on the body weight gain and food intake. Marked effect of body weight gain post treatment of obese rats with VLPE in the different animal groups. The control group showed a mean weight gain of 53.33%. High fat diet increased this value significantly by 235.71%. Treatment with standard drug Orlistat and grape leaves extract significantly decreased the weight gain by 26.52 and 29.77%, respectively, despite the higher food intake**.** Additionally, the food intake of animals in the four groups was different significantly, as shown in **Table 3**.

Table 3Effectof *VLPE on* percent body weight gain and food intake in obese rats induced by HFD

Parameter	control/ND	control/ND/ext	HFD	HFD/ext	HFD/OR
Initial Body Weight (g)	135.00^{a} ± 6.88	137.00 ^{a} ± 3.40	155.00 ^{a} ± 7.32	364.00 ^{b}± 8.45	362.00 ± ^{b}11.88
Final Body Weight (g)	207. 00^{c} ± 9.65	159.00 ^{c} ± 4.09	364.00 ^{c} ± 9.55	250.00^{e} ± 8.26	266.00 ^{e} ± 9.10
Body weight gain (%)	53.33^{i} ±8.23	16.05^{i} ±1.00	+235.71^{f}±7.65	-29.77^{j}± 4.22	-26.52^{j}± 5.51
Food Intake (g/week)	195.00^{a} ± 8.22	199.00 ^{a} ± 5.76	160.00 ^{b} ±6.50	200.00^{a} ± 6.97	202.00^{a} ± 3.00

ND: normal diet, ND/ext: rats feed normal diet and treated orally with *Vitis vinifera* extract for 6 weeks. HFD/ext: rats feed with high fat diet for 12 weeks and treated orally with *Vitis vinifera* for 6 weeks post induction. HFD/OR: rats feed HFD and treated orally for 6 weeks with standard drug Orlistat

3.2. Effect of VLPE on glucose, leptin levels and lipase activity in HFD induced obesity.

Vitis vinifera extract statistically lowered the glucose, lipase levels, as well as leptin enzyme as compared to high fat diet group. With respect to the percentages of improvement, obese rats treated with *Vitis vinifera* extract showed improvement percentage 21.80%, 87.41% and 101.84% in glucose, lipase levels, as well as leptin enzyme, respectively as shown in **Table 4**.

3.3. Effect of VLPE on lipid profile in obese rats for 6 weeks of treatment post induction with HFD

Administration of *Vitis vinifera* extract to high fat diet induced obese rats resulted in significant decrease of total cholesterol (TC), low density lipoprotein (LDL-C) and triglyceride levels (TG) by 16.28, 33.00 and 35.99% respectively, while significant elevation in high density lipoprotein (HDL-C) by 25.81%, as compared to high fat diet group. With respect to the percentages of improvement, obese rats treated with VLPE showed improvement percentage in total cholesterol, HDL-C, triglyceride and LDL-C by 62.01%, 56.75, 54.49% and 46.00%, respectively as shown in **Table 5**.

3.4. Effect of VLPE on liver function enzymes in obese rats for 6 weeks of treatment post induction with HFD

Significant elevation of ALT, AST, ALP and γ GT levels in obese rats by 80.00, 61.42, 93.76 and 89.66% respectively, as compared to normal control rats. while significant decrease was shown in the previous parameters after 6 weeks of treatment with *VLPE* post induction with HFD by 10.00,35.09,23.03 and 3.10% respectively, as compared to high fat diet group as shown in **Table 6**. The obese rats treated with VLPE showed improvement percentage in ALT, AST, ALP and γ GT levels by 70.00, 26.33, 70.73 and 86.55% respectively, as shown in Table 6.

Table 4 Effect of *VLPE on* glucose, leptin levels and lipase activity in HFD induced obesity.

Parameter	ND	ND/ext	HFD	HFD/ext	HFD/OR
Glucose(mg/dl) % change %improvement	89.20[a] ± 7.44	83.22 [a] ± 4.11 6.70	150.00 [b] ± 8.11 68.16	130.55[c] ± 4.10 46.36 21.80	131.00 [c] ±3.87 46.86 21.30
Lipase (UL) change % %improvement	13.11[a] ± 1.92	13.32 [a] ± 1.21 1.60	26.00 [b] ± 2.00 98.32	14.54[a] ± 1.00 10.90 87.41	11.23[a]±1.11 14.34 112.66
Leptin(ng/ml) change % %improvement	4.90[a] ± 0.13	4.00 [a] ± 0.24 18.37	13.00[b] ± 0.57 165.31	8.01 [c] ± 0.99 63.47 101.84	6.87 [d] ± 0.96 40.20 125.10

ND: normal diet, ND/ext: rats feed normal diet and treated orally with *Vitis vinifera* extract for 6 weeks .HFD/ext: rats feed with high fat diet for 12 weeks and treated orally with *Vitis vinifera* for 6 weeks post induction. HFD/OR: rats feed HFD and treated orally for 6weeks with standard drug Orlistat.

Table (5): Effect of *VLPE on* lipid profile in obese rats for 6 weeks of treatment post induction with HFD

Parameter	control /ND	control/ND/ext	HFD	HFD/ext	HFD/OR
TC (mg/dl)% change% improvement	[a]129.00±8.11	[a]100.00±3.00 22.48	[b]230.99±10.00 79.06	[d]150.00±6.00 16.28 62.01	[e]145.80±.7.00 13.02 66.04
HDL-C(mg /dl) % change % improvement)	[a]32.00 ± 3.24	[b]46.00±5.15 43.75	[c]22.10 ±2.14 30.94	[d] 40.26 ±9.40 25.81 56.75	[e] 30.00± 5.23 6.67 24.69
TG (mg/dl) % change % improvement	[a]90.30±7.12	[a]83.00±5.90 8.08	[b]162.00±8.00 79.40	[d]122.80±4.22 35.99 54.49	[a]120.00±2.76 32.89 46.51
LDL-C (mg/dl) % change % improvement	[a]100.00±6.10	[a] 100.00±4.00 0.00	[b]179.00±9.50 79.00	[a]133.00±6.70 33.00 46.00	[c]129.00±9.00 29.00 50.00

ND: normal diet, ND/ext: rats feed normal diet and treated orally with *Vitis vinifera* extract for 6 weeks. HFD/ext: rats feed with high fat diet for 12 weeks and treated orally with *Vitis vinifera* extract for 6 weeks post induction. HFD/OR: rats feed HFD and treated orally for 6 weeks with standard drug Orlistat.

3.5. Effect of VLPE *on apelin, oxidative damage and apoptotic markers in obese rats*

High fat diet statistically lowered the apelin, PON1 and reduced glutathione levels by 65.79, 45.50 and 69.73% respectively, as compared to normal control group. In addition HFD caused significant increase in MDA level by 156.90% respectively, as compared to normal control rats. *VLPE* shown significant increase in apelin, PON and reduced glutathione levels after 6 weeks of treatment by 42.37, 5.86 and 25.33% respectively, and significant decrease in MDA level by 20.69% as compared to high fat diet group. The obese rats treated with VLPE showed improvement percentage in apelin, PON, MDA and GSH by 23.42, 39.64, 136.21 and 44.40% respectively, as shown in **Table 7**.

3.6. Effect of VLPE on inflammatory and apoptotic markers in obese rats

Furthermore, significant elevation in NFKB as well as MCP1 levels by 272.88 and 124.97% respectively, while significant decrease in BCl_2 level by 62.14% was shown in obese rats as compared to normal control rats, while administration of *VLPE* to obese rats, showed significant decrease in NFKB as well as MCP1 levels by 133.90 and 47.36% respectively, while significant elevation in BCl_2 level by 36.43% as compared to high fat diet group as shown in **Table 8.** With respect to improvement percentage, the obese rats treated with VLPE showed improvement percentage in NFKB, BCL_2 and MCP1 by 138.98, 25.71, and 77.62% respectively, **(Table 8).**

Table 6 Effect of *VLPE* on liver function enzymes in obese rats for 6 weeks of treatment post induction with HFD

Parameter	Control/ ND	Control/ ND /ext	HFD	HFD/ ext	HFD/ OR
ALT (U/l)	[a]50.00±3.82	[a]55.70±2.98	[b]90.00±8.00	[a]55.00±2.43	[a]56.80±5.00
% change		11.40	80.00	10.00	13.60
% improvement				70.00	66.40
AST (U/l)	[a]43.00±4.00	[a]41.00±3.65	[b]69.41±7.20	[a]58. 09±5.22	[c]59.65±5.65
% change		4.65	61.42	35.09	38.72
% improvement				26.33	22.70
γ GT (U/l)	[a]29.00±1.00	[a]23.00±1.22	[b]55.00±2.00	[c]29.90±1.22	[a]26.06±0.79
% change		20.69	89.66	3.10	10.14
% improvement				86.55	99.80
ALP (U/l)	[a]69.90±4.00	[a]72.90±4.42	[b]135.44±5.10	[a]86.00±7.22	[a]89.00±4.15
% change		4.29	93.76	23.03	27.32
% improvement				70.73	66.44

ND: normal diet, ND/ext: rats feed normal diet and treated orally with *Vitis vinifera* extract for 6 weeks. HFD/ext: rats feed with high fat diet for 12 weeks and treated orally with *Vitis vinifera* extract 6 weeks post induction. HFD/OR: rats feed HFD and treated orally for 6 weeks with standard drug Orlistat.

Table 7: Effect of *VLPE* on apelin and oxidative damage markers in obese rats

Biomarkers / Groups	Control /ND	Control /ND /ext	HFD	HFDext	HFD/OR
Apelin (ng/l)	[k]380.00±20.00	[k]388.00±25.00	[l]130.00±11.80	[h]219.00±9.80	[d]250.00±11.00
% change		2.11	65.79	42.37	10.71
% improvement				23.42	42.86
PON1 (kU/l)	[i]222.00±10.22	[i]227.00±8.00	[f]121.00±5.823	[j]209.00±6.230	[a] 198.00±8.12
% change		2.25	45.50	5.86	10.81
% improvement				39.64	34.68
MDA (umol/mg protein)	[a]0.58 ± 0.20	[a]0.55±0.09	[h]1.49±0.60	[g]0.70±0.06	[g]0.79±0.04
% change		5.17	156.90	20.69	36.21
% improvement				136.21	120.70
GSH (ug/mg protein)	[a]49.55 ± 6.20	[a]59.00 ± 4.29	[b]15.00 ± 1.00	[c]37.00 ± 3.00	[d]26.00 ± 2.00
% change		19.07	69.73	25.33	47.53
% improvement				44.40	22.20

ND: normal diet, ND/ext: rats feed normal diet and treated orally with *Vitis vinifera* extract for 6 weeks. HFD/ext: rats feed with high fat diet for 12 weeks and treated orally with *Vitis vinifera* extract for 6weeks post induction. HFD/OR: rats feed HFD and treated orally for 6 weeks with standard drug Orlistat.

Table 7 : Effect of *VLPE* on inflammatory and apoptotic markers in obese rats

Biomarkers / Groups	Control /ND	Control /ND /ext	HFD	HFD/ ext	HFD/OR
NFkB (U/l) % change % improvement	[a]59.00±4 .34	[a]58.00±8.01 1.69	[b]220.00±20.00 272.88	[c]138.00±5.10 133.90 138.98	[d]159.00±6.08 169.49 103.39
BCl_2 (μg/l) % change % improvement	[f]14.00±1.12	[f]13.65±1.00 2.5	[e]5.30± 0.40 62.14	[a]8.90±0.64 36.43 25.71	[a]7.43±0.94 46.93 15.21
MCP1(Pg/ml) % change % improvement	[a]8.89±0.90	[a]8.90±1.00 0.11	[h]20.00±0.90 124.97	[c]13.10±0.99 47.36 77.62	[d]17.90±1.08 101.35 23.62

ND: normal diet, ND/DS: rats feed normal diet and treated orally with *Vitis vinifera* extract for 6weeks .HFD/ext: rats feed with high fat diet for 12 weeks and treated orally with *Vitis vinifera* extract for 6 weeks post induction. HFD/OR: rats feed HFD and treated orally for 6 weeks with standard drug Orlistat.

3.7. Histopathological examination

3.7.1. Histopathology investigation of the blood vessels

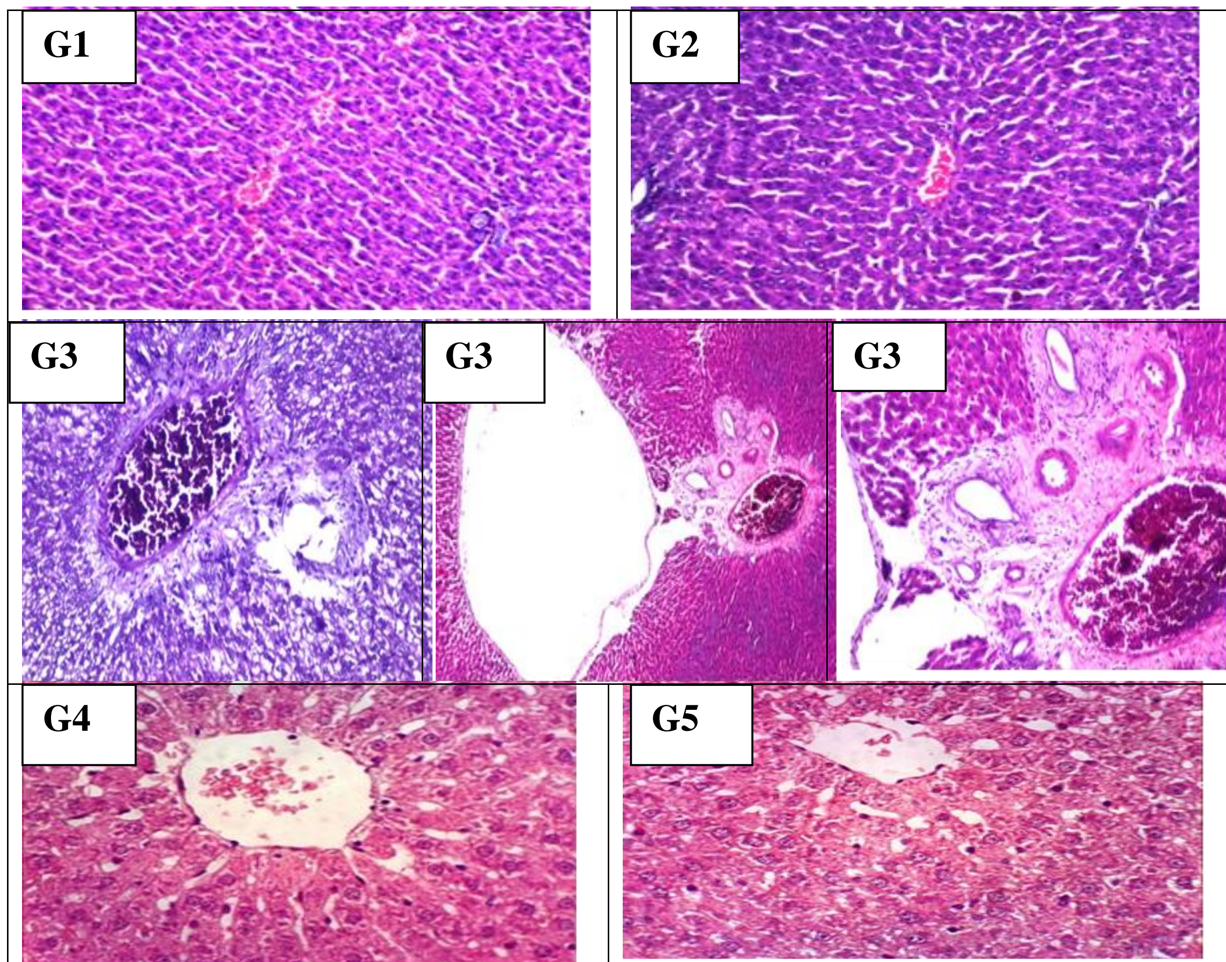

Fig. 1: Photomicrograph of blood vessels in different therapeutic groups.
G1and G2: The histology of blood vessels of normal control rats and control rats treated with Vitis vinifera extract showed no histopathological alteration and the normal histological structure of the central vein and surrounding hepatocytes in the parenchyma.
G3: The histology of blood vessels of obese rats showed multiple intracytoplasmic micro fat vacuoles in the hepatocytes as fatty change associated with inflammatory cells infiltration in the portal area and sever dilatation and congestion in the portal vein.
G4: The histology of blood vessels of obese post treatment with *Vitis vinifera* showed the normal histological structure of hepatic lobule with mild inflammatory cells infiltration in the portal area.
G5: The histology of blood vessels of high fat diet rats post treatment with Orlistat reference drug showed normal histological structure of hepatic lobule with normal histological structure of the central vein.

3.7.2. Histopathological examination of the kidney tissue

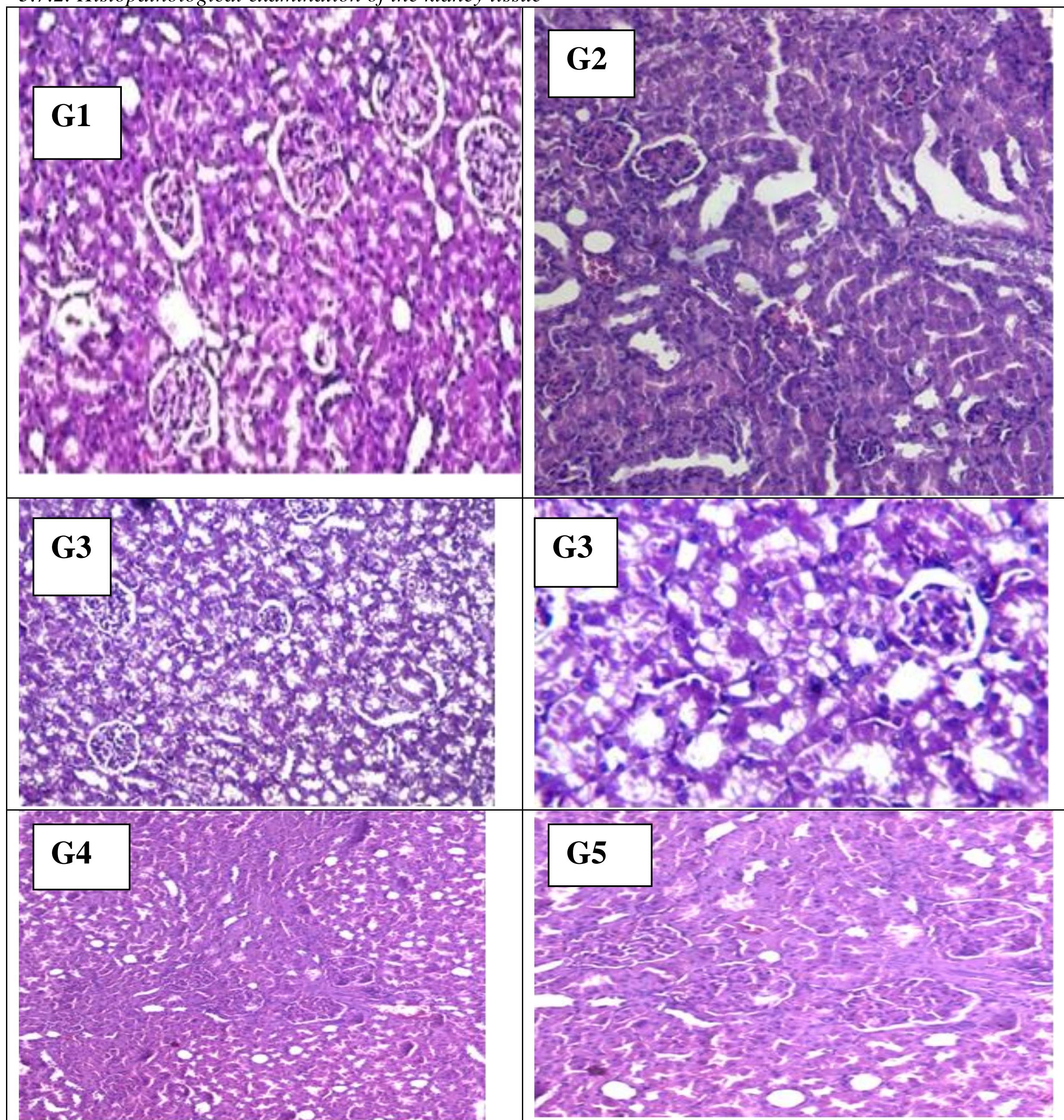

Fig. 2 Photomicrograph of kidney tissue in different therapeutic groups.
G1 and G2: Histopathology of the kidney of normal control rats and control rats treated with Vitis vinifera extract showed no histopathological alteration and normal histological structure of the glomeruli and tubules at the cortex.
G3: kidney of obese rats showed vacuolar degeneration in the epithelial cells lining the tubules.G4 and G5: kidney of obese treated with Vitis vinifera extract and Orlistat drug showed no vascular congestion no inflammation.

4. Discussion

One therapeutic plants may apply promising pharmacological properties and improve the adequacy of traditional prescriptions as integral specialists **[49]**. Perhaps the most burned-through organic products all around the world is *Vitis vinifera* (Grape). *V. vinifera* has a wide scope of pharmacological exercises because of its rich polyphenol fixings **[50]**.

The anti-hypercholesterolemic and antiobestic efficacy of different doses of methanol and aqueous extracts of *V. vinifera* leaves were evaluated on high cholesterol diet rat which significantly attenuated lipid levels **[17]**. Moreover, the methanol extract was more effective than aqueous extract. On the other hand, the leaves ethanolic extract of *V. vinifera* using **dose 200 mg/kg** had a noticeable antidiabetic effect and antioxidant activity on streptozotocin-induced

diabetic rats in addition it protected liver and kidney tissues from the oxidative damage , increased the GSH and PON1 content in liver tissue **[51]**.

In the current study, the anti-obesity efficacy of VLPE may be contributed to phytoconstituent content including; phytol, phytosterol, lupeol, fatty alcohols and unsaturated fatty acids.

Phytol supplementation diminished body weight gain and index of inguinal subcutaneous white fat tissue (iWAT), and enhanced the browning of iWAT of mice, with the elevation of brown adipocyte genes expression (UCP1, PRDM16, PGC1α, PDH, and Cyto C). Also, it stimulated signaling pathway of AMPKα in iWAT of mice **[52]**. Another study showed that phytol as a promising agent for nutrition to overcome obesity and type 2 diabetes through increased number of adipocyte in iWAT with the smaller average adipocyte and improved glucose tolerance in mice fed HFD. Also, it could markedly increase genes expression linked with adipogenesis (PPARγ and C/EBPα), glucose uptake (AS160 and GLUT4), and was associated with activation of PI3K/Akt signaling pathway **[53]**. On the other hand, phytol stimulates PPAR-α in the hepatic and brown fat tissue in high fat diet-induced severe obese mice that to ameliorate obesity-induced metabolic abnormalities **[54]**. Moreover, phytol as a cholesterol lowering agent, it can be administered clinically to patients with type II diabetes, obesity or other patients in risk of cardiovascular diseases due to elevated cholesterol or triglycerides (patent/WO2009113952A1). In the present study, obese rats declared a noticeable increase in the level of glucose, while a significant reduction in its level in VLPE treated obese rats relative to obese untreated one .Many factors clarify increased level of blood glucose in obesity, such as decreased insulin sensitivity, attenuated pancreatic lipase and cell function, as well as augmented hepatic gluconeogenesis **[55,56]**. Feeding of excessive cholesterol induces hypercholesterolemia and arteriosclerosis and further elicits the progress of obesity and dyslipidemia in both humans and rodents by changing the levels of plasma cholesterol and triglyceride **[57]**. Except the level of HDL-c, the level of lipids was found to be significantly increased in rats of *Vitis vinifera* leaves produced a marked amelioration in lipid level. While, significant decrease in HDL level in obese rats relative to control. This was recovered by supplementation of *Vitis vinifera* leaves that significantly increased ($p < 0.05$) the level of HDL-c post treatment. *Vitis vinifera* leaves extract could effectively reduce the level of cholesterol and ameliorated HDL level in atherosclerosis rats. Also, the VLPE supplementation protected endothelial and thickness of blood vessel linings in rodents. V*itis vinifera* acts *as* anti-hypercholesterolemic and antiobestic agent due to presence of active phyto-principles constituents and antioxidants components. The damaging effect of high fat diet on the AST, ALT, ALP, GGT and lipid profile as well as increased liver MDA levels was prevented by the Vitis extract compared to standard Orlistate drug. Cytosolic enzymes are leaked into the blood stream, when there is damage to the liver cell membrane. Thus, the increase of blood cytosolic enzymes is a promising quantitative biomarker to determine the extent of hepatic destruction and alteration in membrane permeability **[58, 59]**. The elevated levels of ASP, ALT, ALP and GGT were attenuated by treatment with the *V. vinifera* extract. The protective effect of Vitis leaves t against oxidative damage was evaluated by measuring GSH and catalase levels. Our study declared a significant reduction in the levels of lipid peroxidation with noticeable increase in reduced glutathione in liver of *V. vinifera* treated rats **[9]**. These results recommended the attenuated role of *V. vinifera* (VLPE) in oxidative destruction of hepatic tissue in obese treated rats **[60]**. Our results are consistent with earlier studies, which strongly suggest that *V. vinifera* may protect the structural integrity of hepatocytes and prevent the leakage of cytosolic enzymes into blood stream. **[60]**.

The mixture of long-chain aliphatic alcohols (Policosanol) mainly composed of octacosanol (63%) lowered clinically total and low-density lipoprotein (LDL-c) cholesterol and raised high-density lipoprotein (HDL-c) cholesterol levels **[61]**. Concerning the phytosterol content, **Burdziej et al.[18]**, found that the total phytosterols content of all tested grapevines leaves ranged from 0.6 to 1.7 mg/g of dry leaf weight and β-sitosterol was the most abundant phytosterol of all tested leaves. According to the European Food Safety Authority (EFSA), consumption of 2 – 2.4 g/day of phytosterols consistently reduced cholesterol absorption and circulating low-density lipoprotein (LDL-c)-cholesterol by nearly 9%, active in reducing serum cholesterol **[62]**.

The pentacyclic triterpenoids (ursane-, oleanane-, lupane- and friedooleanane (taraxerane)-type skeletons, i.e. α-amyrin β-amyrin , lupeol and taraxerol) content in grape leaves cultivars ranged

from 0.36 mg/g to 1.5 mg/g of dry leaf weight (Burdziej,et al 2019)(18). The total triterpenoids level decreases gradually during fruit development, with an increase in the neutral triterpenoid level **[63]**. **Pensec et al. [17, 63],** classified pentacyclic triterpenoids, of eight Upper Rhine Valley cultivars and numerous domesticated grapevines leaves into lupeol- or taraxerol-rich groups. Recent studies demonstrated that consumption of the preparation of triterpene alcohol and sterol from rice bran avoid high-fat diet-induced obesity and decrease postprandial blood glucose in mice **[64]**. By enhancing fatty acid oxidation in muscles and lowering fatty acid synthesis in the liver through GIP-dependent and GIP-independent mechanisms **[65]**. The administration of triterpenoids and sterols block the ability of inflammatory cytokines such as interferon gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) to induce transcription of the iNOS gene **[66]**.

Several studies showed that lupeol (50 mg/kg) could reduce levels of triglycerides, glycerol and cholesterol (LDL) as well as cholesterol (HDL) levels in dyslipidemic animals' hamster **[67].).** Also, it exhibited antihyperglycemic action and its organization bring down the danger of advancement of diabetes in creature models. Lupeol repressed the action of protein tyrosine phosphatase 1B (PTP1B) which assumes a significant part in the hindrance of insulin activity, improvement of type 2 diabetes and weight **[68]**. (In addition, it fu ndamentally lightened the liver capacity irregularities and expanded fecal discharge of cholesterol **[69]**.

Docking studies of phytosterols and triterpenoids *via* interaction with enzymes related to inflammation, such as MPO; PLA2, *i*-NOS; LOX-5; COX-1 and -2 indicates that pentacyclic skeletons exhibited low theoretical enzyme binding while tetracyclic skeletons exhibited better binding scores. However, triterpenoids skeleton may have an affinity to several enzymes involved in the inflammatory process **[70]**.

By molecular docking using BINDSURF, 47 of triterpenes and sterols identified in *Morinda lucida* Benth (*Rubiaceae*) were screened for their inhibitory activities against BCL-XL, BCL-2, MCP, NK_KB and MCL-1. The result revealed that ursolic acid, oleanolic acid, cycloartenol, campesterol, stigmasterol and β-*sitosterol* have higher binding affinities for the selected BCL-2 proteins, compared to known standard inhibitors **[71]**.

VLPE contain ω-6/ω-3 in ratio (1:2) where dietetics recommended lowering the ω-6/ω-3 ratio for maintenance of health and reducing the risk of disease **[72]**. The occurrence of the polyunsaturated fatty acids particularly ω-3 linolenic acid (11.49%) which is essential for the synthesis of n-3 PUFAin mammals. n-3 PUFA can minimize the obesity-induced metabolic disorders incidence , including insulin resistance, hypertension and dyslipidemia **[73]**. Also, **Huang et al. [73]**, discussed the beneficial effects of n-3 PUFA in adipocyte regulation, apelin and leptin levels as well as lipase activity through lipid metabolism, energy expenditure, and inflammation.

5. Conclusion

In this way, plant might be a wellspring of bioactive lipids that might be supplied in food, referred as cholesterol-bringing down diminishing the danger of obesity complication

6. Funding

Funded by authors

7. Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

8. Acknowledgements

Declared none.

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