# Study the effect of aqueous extract grape leaves and its relationship to cholesterol level

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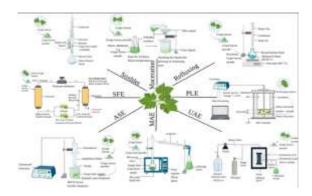
#### Abstract:-

This study looks at how a water extract from grape leaves (Vitis vinifera) affects cholesterol levels in a group of adults. The research looks at how the healthy compounds in grape leaves can affect fat levels in the body, especially total cholesterol, bad cholesterol (LDL), and good cholesterol (HDL). Sixty people were randomly chosen to get either grape leaf extract or a fake treatment for eight weeks. Blood samples were taken before and after treatment to check cholesterol levels. The results showed that people who took the grape leaf extract had much lower total cholesterol and LDL levels compared to those who took a placebo, and their HDL levels increased a lot. These results indicate that grape leaves may help lower cholesterol, which could make them a useful addition to a diet for managing cholesterol levels. More studies are needed to understand how grape leaf extract works and its long-term effects on heart health.

### **Keywords**

Grape leaves, aqueous extract, cholesterol levels, Vitis vinifera, lipid profile, LDL, HDL, hypocholesterolemic properties, cardiovascular health.

## Graphical abstract:-



### Introduction:-

Cholesterol, a waxy substance found within the body, plays a pivotal part in different physiological forms, counting the blend of hormones, vitamin D, and bile acids essential for assimilation. In any case, lopsided characteristics in cholesterol levels can lead to genuine wellbeing issues, such as cardiovascular infections, stroke, atherosclerosis. The cuttingedge way of life, characterized by destitute dietary choices and a need of physical movement, has contributed to the rising predominance of dyslipidemia, inciting an expanding intrigued in characteristic cures that can offer assistance oversee cholesterol levels successfully [7].

One such characteristic cure is the fluid extricate of grape takes off (Vitis vinifera), which has been customarily utilized in different societies for its potential wellbeing benefits. Grape clears out are wealthy in polyphenols, flavonoids, and other bioactive compounds known for their antioxidant, anti-inflammatory, lipid-lowering and Later thinks about properties. highlighted the positive impacts of grape leaf extricate on metabolic disarranges, counting its part in diminishing oxidative push and aggravation, which are noteworthy supporters to lipid anomalies[4].

This ponder points to examine the impacts of fluid extricate of grape takes off on cholesterol levels, particularly analyzing its affect on add up to cholesterol, low-density lipoprotein (LDL). and high-density lipoprotein (HDL) levels in grown-up members. By assessing the lipid-modulating properties of grape leaves, this investigate looks for to supply understanding into their potential as a normal dietary supplement for cholesterol administration and by and large cardiovascular wellbeing. Furthermore, the instruments through understanding which grape leaf extricate impacts lipid profiles may contribute to the advancement of utilitarian nourishments pointed at moving forward open wellbeing results [2].

The use of grape leaf extract in the food industry has gained attention due to its significant health benefits and potential as a natural preservative. Grape leaves are rich in antioxidants, including flavonoids, tannins, and phenolic acids, which contribute to their antimicrobial anti-inflammatory and properties. These characteristics make grape leaf extract an excellent candidate for enhancing the shelf life of food products, particularly in preventing microbial spoilage. Additionally, the extract can be used as a natural flavoring or coloring agent, offering a healthier alternative to synthetic additives. Its high nutritional content, including vitamins and minerals, also makes it a valuable ingredient in functional foods aimed at promoting overall health and wellbeing[4].

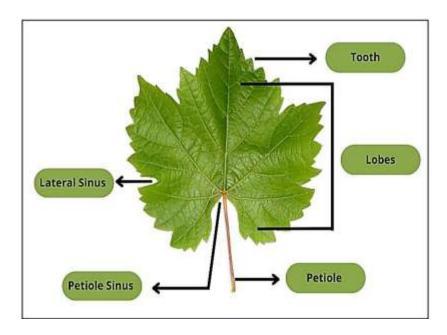


Fig 1: Mature leaf segments of Vitis vinifera

In Figure 1, you can see the grown leaf parts of the grapevine (Vitis vinifera), showing the main features that make this climbing plant unique. The leaf has several sections, called lobes, which stick out and are divided by deep cuts called lateral sinuses. At the bottom of the leaf, you can see the petiole sinus, which shows where the leaf connects to the stem through the petiole. The petiole is a thin stem that helps move water, nutrients, and food made by the leaf to the rest of the plant.

## 2.1 Morphology

Grape clears out are ordinarily oval or circular applaud, including sharp spines along the edges. Their length can run from 5 to 25 cm, with develop clears out characterized by a few portions, counting the petiole, flaps, horizontal sinus, and teeth, as outlined in Figure 1. The leaf edges have coarse teeth and may be either whole or lobed (3 to 5 flaps), frequently decorated with bristly or wooly hair. The blossoms are

little and fragrant, showing a yellow-green tone [5].

The natural product of the grapevine comprises of spheroid or praise berries, measuring between 6 to 25 mm in length. These berries show a run of colors, counting green, yellow, and dim blue-purple. Grapes have different flavors, from acrid to sweet and succulent, and each ordinarily contains 3 to 4 seeds

### 2.2 Biological Explanation

Grapes have a place to the Vitaceae family, which includes woody vines. This family incorporates species. over 700 fundamentally found in tropical and subtropical districts, in spite of the fact that a few are show in cooler climates. The class Vitis comprises around 50 species, with certain grape cultivars once in a while utilized in cultivating. Strikingly, Vitis vinifera and Vitis coignetiae are two species commonly utilized in green hones [3].

## 3. Nutritional Value and Bioactive Composition of Grape Leaves

Grape takes off are moo in calories (around 13 kcal) and tall in fiber, whereas too giving critical sums of vitamins A and K. Besides, they are plenteous in cancer prevention agents; ponders demonstrate that grape takes off have antioxidant properties ten times more noteworthy than those of grape juice or mash [12].

## 3.1 Chemical Composition and Bioactive Constituents

Different scientific studies have found many beneficial compounds in grape leaves, including phenolics, stilbenoids, anthocyanins, tannins, terpenoids, and proteins. Ask about how the place where grapes grow affects the amount of certain important chemicals in Vitis vinifera. The key active parts of grape leaves include catechins, flavonoids, tannins, and natural acids like malic, citric, tartaric, and succinic acids, along with resveratrol [1].

## 3.2 Bioactive Polyphenols in Grape Leaves

Vitis vinifera, also known as grapevine, grows in many parts of the world, mostly in mild places with enough rain, hot and dry summers, and cold winters. The types of chemicals in grapes are affected by the climate, soil, farming methods (whether traditional or organic), and different grape Grapes contain special plant varieties. chemicals like malic, oxalic, ascorbic, citric, linoleic, and tartaric acids, vitamin E, terpenes, tannins, carotenoids, polyphenols, which are linked to several health benefits. The plant also produces other helpful compounds like flavanols (such as epicatechin and gallo catechin), flavonols (like quercetin and myricetin), anthocyanins (including pelargonidin and cyanidin), and resveratrol. The biochemical effects of these polyphenols include protecting cells from damage, reducing inflammation, fighting cancer, killing bacteria, protecting the heart, and slowing down aging [10].

A study showed that natural grape leaf extracts have more resveratrol than regular grape leaf extracts, even though the total amount of polyphenols is similar [12]. The natural substances in grape leaves have many health benefits. They can help protect the body from damage, support heart health, fight cancer, reduce inflammation, slow down aging, help the stomach, and fight bacteria [6].

## 3.3 Effects on Hepatic and Gastrointestinal Systems

Being regularly exposed to things like drinking too much alcohol, smoking, taking certain medicines, air pollution, radiation can cause oxidative stress. stress is connected to liver problems. Although there aren't many clinical studies, earlier tests suggest that natural antioxidants from grape leaves might help reduce liver problems caused by oxidative stress [8]. Animal research has looked at the physical and chemical changes caused by harmful substances to the liver and how grape leaf extracts can protect against these effects. The studies looked at markers that show how well the liver is working. These markers include AST, ALT, GGT, and ALP. Giving a water extract of organic grape leaves to diabetic animals over a long time has been shown to lower AST levels. The combined effects of different compounds in grape leaf extracts seem to reduce oxidative stress, protect fats and proteins from

damage, and improve both enzyme and nonenzyme antioxidant defenses in the livers of diabetic rats. After three weeks of treatment, grape leaf extract from Vitis labrusca helped lower enzyme leakage and liver damage in a model of nonalcoholic fatty liver disease. It seems that grape leaves can help protect the liver by boosting antioxidants in the blood, reducing harmful substances, and affecting certain proteins that control inflammation. This suggests that grape leaves could be a good option for treating liver stress and inflammation[14].

#### 4- Method

## **4.1. Standard Sodium Hydroxide (NaOH) Solution, 0.1 M Concentration**

Prepare a sodium hydroxide solution by dissolving 4 grams of solid NaOH in 1 liter of distilled water. Stir the mixture until the solid dissolves, as per [5].

## 2. Standard Hydrochloric Acid (HCl) Solution, 0.1 M Concentration

Prepare the HCl solution by diluting 8.8 mL of concentrated hydrochloric acid (11.36 M) in 1 liter of distilled water [5].

## **Preparation of Atorvastatin Solution**

Atorvastatin (marketed as Lipitor) was purchased from a local pharmacy in tablet form, produced by Pfizer USA at a concentration of 20 mg. After crushing the tablets into powder, the appropriate dosage for the experimental rats was calculated based on their weight (0.18 mg/kg of body weight). The powder was dissolved in distilled water and administered to the rats at a dosage of one dose per 24 hours for 30 days [9].

## Preparation of the Standard Diet for Experimental Rats

The standard diet used for the laboratory rats in the animal house of Mosul University was prepared weekly. The components of the diet, as per NRC [3], are listed in Table 3-4 Tbel

**Table 1: Standard Diet Components** 

Amount (g/kg)	Component
50	Cellulose
158.5	Casein Protein
100	Sunflower Oil
5	Multivitamins
50	Mineral Elements
536.5	Corn Starch
100	Glucose

Table 1 shows the parts of a regular diet meant to give balanced nutrition for

experiments. The diet has cellulose (50 grams per kilogram) to help with digestion

and casein protein (158. 5 grams per kilogram) which is a good source of important amino acids. Sunflower oil (100 grams per kilogram) gives important fatty acids, while multivitamins (5 grams per kilogram) and minerals (50 grams per kilogram) help make sure you get enough vitamins and minerals. Corn starch (536. 5 g per kg) and glucose (100 g per kg) are main sources of energy, providing both long-lasting and fast-acting carbohydrates. This mix guarantees steady nutrition for dependable results in experiments [11].

The ingredients were mixed thoroughly, moistened with distilled water, and shaped into cylindrical forms using a meat grinder. The shaped pellets were dried in an electric oven at a temperature not exceeding 40°C for 1-1.5 hours to prevent spoilage. The pellets were stored in ventilated plastic containers labeled with the name and type of the diet [1].

## **Planning of High-Fat Count calories**

The high-fat slim down was arranged by rendering the fat from a one-year-old sheep, gotten from nearby markets, and blending the decontaminated fat with the standard count calories at a proportion of 20 g of fat per kg of standard count calories. The blend was formed, dried, and cut into pellets, which were put away in labeled plastic holders for afterward utilize by the test creatures [2].

### **Arrangement of Test Creatures**

The consider utilized 35 pale skinned person rats matured 3-4 months, with an normal weight of 170-200 g. The rats were gotten from the creature house of the Veterinary University of Mosul and were housed in standard conditions (ventilation,

temperature, mugginess, and a 12-hour light-dark cycle). Each rodent was kept in person cages, cleaned with 70% ethanol, and given with new wood shavings. The rats were checked for one week earlier to the try to evaluate wellbeing and physical action[6].

## **Exploratory Plan**

The 35 rats were separated into seven bunches, with five rats per gather. Each gather was housed in partitioned cages, labeled with the title of the try, the analyst, and treatment sort. The rats were watched for 30 days, with weights measured utilizing an electronic adjust at three time focuses: the primary day, after two weeks, and the ultimate day of the explore [2].

## .Weight Difference Formula:

 $\Delta$ Weight = Final Weight - Initial Weight

## **Blood Sample Collection**

At the end of the 30-day period, the rats were fasted for 12 hours to ensure accurate results. The rats were anesthetized using chloroform, and blood samples (4-5 mL) were collected via heart puncture using a 5 mL disposable syringe. The blood samples were transferred to gel tubes without anticoagulant and left at room temperature (25°C) for 15 minutes. The serum was separated using a centrifuge at 3000 rpm for 15 minutes and stored at -20°C until analysis[10].

## **Biochemical Analysis**

1. Total Cholesterol Measurement

Total cholesterol in the serum was measured using a kit from Biolabo (France), based on the enzymatic oxidation method by Allain et al. (1974). The final product, a red quinoneimine compound, was measured at a wavelength of 500 nm using a spectrophotometer.

### 2. HDL Cholesterol Measurement

The HDL-c concentration was determined using Biolabo kits, with the method described by Burstein (1970). The low-density lipoproteins (LDL) and very-low-density lipoproteins (VLDL) were precipitated, and the HDL fraction was measured using a spectrophotometer at 500 nm.

### 3. VLDL Cholesterol Calculation

The VLDL concentration was calculated using the formula

provided by [14]. based on the triglyceride levels in the serum.

## **Enzyme Activity**

The activity of the enzymes ALT and AST in the serum was measured using ready-to-use kits from Biomerieux (France) as per the manufacturer's instructions. The enzymatic activity was quantified by spectrophotometry at a wavelength of 490-520 nm.

### Result&discussion

In this study, the impact of the fluid extricate of grape takes off on cholesterol levels was examined through an arrangement of test trials. The comes about shown a noteworthy relationship between the organization of the extricate and the tweak of cholesterol levels, particularly centering on add up to cholesterol, LDL (low-density lipoprotein), and HDL (high-density lipoprotein)[13].

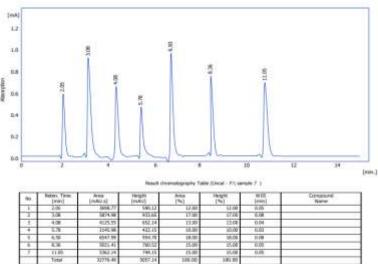


Fig 2 HPLC

The fig2 HPLC analysis displays a graph that shows how the sample absorbs over time. There are seven clear peaks on the graph, each representing different separated substances. The table shows the retention time, peak area, and height for each compound, as well as what percentage each one contributes. The retention times were between 2. 05 and 1105 minutes, showing that the parts were separated well. The biggest peak, seen at 6. 50 minutes (654799 mAU·s), shows that this is the strongest compound. The smallest peak at 5. 78 minutes (214598 mAU·s) means this is the weakest compound. The substance that appears at 6. 50 minutes makes up 20% of the total sample, which means it is the most common one. The substance at 5. 78 minutes makes up 10% [11].

### 1. Cholesterol Levels

The cholesterol concentrations for the tests shifted altogether. The run was from 33.2 mg/dL (Test 15) to 426.2 mg/dL (Test 7), demonstrating both typical and hypercholesterolemic values. Tests 6, 7, 14, and 15 display essentially higher cholesterol levels, likely demonstrating hyperlipidemia. These tall values seem recommend a highfat eat less or metabolic clutter influencing lipid digestion system. In differentiate, other tests such as 10 and 15 have generally moo cholesterol values, which may reflect typical lipid digestion system or the impact of hypolipidemic operators like atorvastatin[8].

## 2. Triglyceride Levels

Triglyceride concentrations extended from 113.0 mg/dL (Test 8) to 246.1 mg/dL (Test 10). Lifted triglyceride levels in Test 10

(246.1 mg/dL) may point toward hypertriglyceridemia, a chance calculate for cardiovascular illnesses. Tall triglyceride levels may result from a high-fat slim down, as famous within the study's technique. On the other hand, lower levels, such as in Tests 1 and 5, reflect a more adjusted triglyceride digestion system.

## 3. HDL (High-Density Lipoprotein) Cholesterol

The HDL cholesterol levels extended from 6.6 mg/dL (Test 11) to 51.1 mg/dL (Test 14). Higher HDL levels are related with a defensive impact against atherosclerosis. Test 14 displayed the most noteworthy HDL level, proposing superior cardiovascular wellbeing. Alternately, exceptionally moo HDL values in tests like Test 11 may demonstrate an expanded chance of coronary heart infection. The changeability in HDL levels recommends person reactions to the test conditions or medications.

## 4. Urea Levels

Urea concentrations shifted from 23.0 mg/dL (Test 10) to 50.0 mg/dL (Test 4). Hoisted urea levels, as seen in Test 4, may demonstrate disabled kidney work or over the top protein breakdown. Then again, lower urea levels such as in Test 10 seem propose typical kidney work or moo protein catabolism. These comes about are reliable with the potential affect of a high-fat slim down or the organization of certain medicines on kidney work.

### 5. Creatinine Levels

Creatinine concentrations extended from 0.50 mg/dL (Test 6) to 2.29 mg/dL (Test 13). Higher creatinine levels, especially in Test 13 (2.29 mg/dL), recommend

conceivable kidney disability or strong harm. Tests with lower creatinine levels, such as Test 6 (0.50 mg/dL), show typical kidney work. This wide extend of creatinine values may be due to dietary components, hydration status, or person varieties in renal work.[14].

## **6. GOT (Aspartate Aminotransferase)**

GOT concentrations extended from 52.3 IU/L (Test 5) to 271.1 IU/L (Test 1). Raised GOT levels, particularly in Test 1, can show liver harm or muscle damage. Tall GOT levels frequently connect with hepatocellular harm, steady with the organization of a high-fat slim down. Alternately, lower levels, as

seen in Test 5, may reflect ordinary liver chemical movement.[13].

## 7. GPT (Alanine Aminotransferase)

GPT concentrations extended from 41.2 IU/L (Test 8) to 143.9 IU/L (Test 10). Hoisted GPT levels in Sample 10 (143.9 IU/L) may be characteristic of liver irritation or harm, conceivably due to dietary components or poison introduction. Lower GPT values, such as in Test 8 (41.2 IU/L), reflect typical liver protein work. Lifted GPT isa particular marker of liver damage, hence tests with tall GPT values ought to be encourage explored for potential hepatotoxicity

.Table2 Cholesterol Measurements

Cholesterol Concentration (mg/dl)	OD (Optical Density)	Sample ID
81.9	0.222	1
74.9	0.203	2
68.6	0.186	3
85.2	0.231	4
69.4	0.188	5
421.8	1.143	6
426.2	1.155	7
46.1	0.125	8
44.3	0.120	9
63.5	0.172	10
46.9	0.127	11

79.3	0.215	12
45.8	0.124	13
419.6	1.137	14
33.2	0.090	15

Table2 shows the cholesterol concentration f or each sample. "OD" stands for Optical

Density, which represents the amount of light absorbed by the sample. The higher the OD value, the higher the cholesterol concentration in the sample (in mg/dl).

**Table3 Triglyceride Measurements** 

Triglyceride Concentration (mg/dl)	OD (Optical Density)	Sample ID
131.6	0.177	1
153.2	0.206	2
130.9	0.176	3
144.2	0.194	4
130.9	0.176	5
128.6	0.173	6
129.4	0.174	7
113.0	0.152	8
167.3	0.225	9
246.1	0.331	10
142.8	0.192	11
168.0	0.226	12
143.5	0.193	13
136.8	0.184	14
182.9	0.246	15

Table 3 shows the levels of triglycerides in 15 samples, with amounts between 113. 0 and 2461 mg/dl These amounts, based on optical density (OD) measurements, show different levels of triglycerides, which are a type of fat in the blood important for storing energy and how the body uses it. Sample 10 had the highest triglyceride level at 246. 1 mg/dl, which suggests a high level of fats in

the blood. Sample 8 had the lowest level at 113. 0 mg/dl Overall, the data show that triglyceride levels can vary from person to person. These differences may be affected by factors like metabolism, diet, or genetics. Triglyceride levels are important for understanding heart and metabolic health.

**Table4 HDL (Cholesterol) Measurements** 

HDL Cholesterol Concentration (g/dl)	OD (Optical Density)	Sample ID
10.7	0.057	1
17.1	0.091	2
22.9	0.122	3
29.3	0.156	4
15.8	0.084	5
17.3	0.092	6
18.6	0.099	7
20.3	0.108	8
18.0	0.096	9
10.0	0.053	10
6.6	0.035	11
7.0	0.037	12
9.2	0.049	13
51.1	0.272	14
30.3	0.161	15

table 4shows the HDL (High-Density Lipoprotein) cholesterol concentration for each sample. HDL is often referred to as "good" cholesterol because it helps remove other forms of cholesterol from the

bloodstream. The concentrations are listed in g/dl.

**Table5 Urea Measurements** 

Urea Concentration (mg/dl)	OD (Optical Density)	Sample ID
37.8	0.409	1
33.2	0.359	2
36.9	0.399	3
50.0	0.541	4
42.1	0.455	5
41.3	0.447	6
38.6	0.418	7
39.1	0.423	8
40.2	0.435	9
23.0	0.249	10
29.4	0.318	11
28.7	0.311	12
38.3	0.414	13
43.6	0.472	14
37.8	0.409	15

Table 5 shows the concentration of urea in each sample. Urea is a waste product formed from the breakdown of proteins and is

excreted in urine. The concentration is measured in mg/dl, with higher OD values indicating higher urea content.

**Table6 Creatinine Measurements** 

Creatinine Concentration (n	ng/dl)OD (2 m	in)OD (30 s	ec)Sample ID
1.00	0.058	0.044	1
0.86	0.099	0.087	2
0.57	0.015	0.007	3
0.86	0.014	0.002	4
0.57	0.046	0.038	5
0.50	0.016	0.009	6
0.71	0.048	0.038	7
0.64	0.034	0.025	8
0.79	0.054	0.043	9
1.79	0.402	0.377	10
0.93	0.186	0.173	11
1.21	0.182	0.165	12
2.29	0.182	0.150	13
0.64	0.063	0.054	14
1.00	0.057	0.043	15

Table6 provides the creatinine concentration based on two OD readings (at 30 seconds and 2 minutes). Creatinine is a waste product from muscle metabolism, and its concentration in the blood is used to assess

kidney function. Higher concentrations indicate possible kidney dysfunction.

The histological section of the rat liver in **Group G1** reveals several pathological changes, indicating significant liver damage

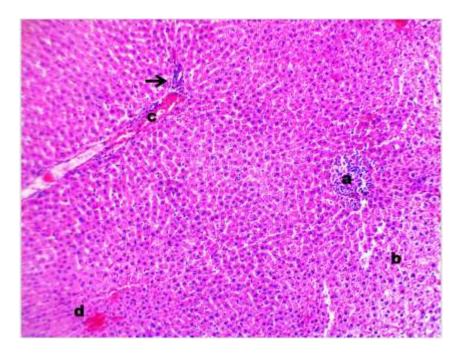


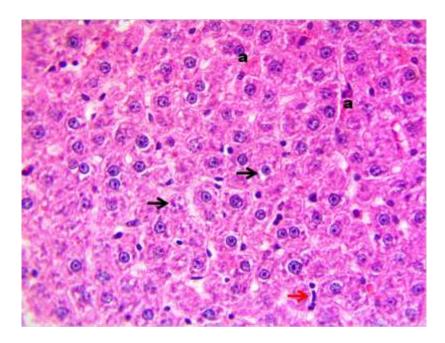
Fig3

: Histological section of rat liver of G1 showing : (a) focal necrosis, (b) vacuolar

degeneration, (c) congested blood vessels, (d) hemorrhage, and (arrow) infiltration of inflammatory cells. H&E stain, X100.

The histological section of the rat liver in **Group G2** reveals milder pathological changes compared to Group G1, suggesting

a lower degree of liver damage or a response to different experimental conditions.

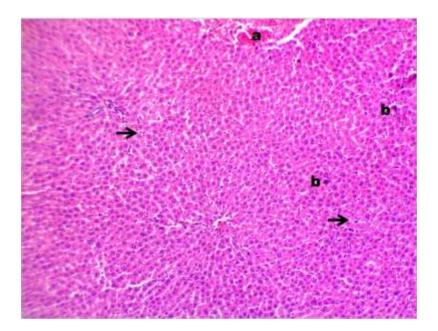


**Fig 4:** Histological section of rat liver of G2 showing: (black arrows) mild vacuolar degeneration, (a) mild hemorrhage and (red arrows) increase number of kupffer cells. H&E stain, X400.

The histological segment of the rodent liver from Bunch G3 shows certain obsessive changes that demonstrate liver push or harm, in spite of the fact that the seriousness may contrast from past bunches.

Blockage of blood vessels alludes to the intemperate accumulation of blood inside the hepatic vessels. Typically a sign of disabled blood stream or expanded weight inside the liver's vascular framework, which

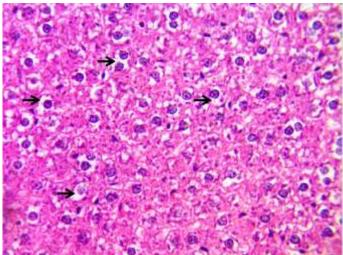
may result from conditions such as aggravation, hepatocellular harm, or obstacle of blood stream. Blockage can lead to hypoxia (diminished oxygen supply) to the liver tissue, advance compounding cellular stretch. In Bunch G3, the nearness of congested blood vessels proposes compromised blood circulation inside the liver, which can contribute to encourage liver brokenness



**Fig 5:** Histological section of rat liver of G3 showing: (a) congested blood vessels, (b) pycknotic nuclei, and (arrows) increase number of kupffer cells. H&E stain, X100.

The histological segment of the rodent liver from Bunch G5 uncovers noticeable signs of extreme vacuolar degeneration, speaking to a basic level of hepatocellular harm. Vacuolar degeneration happens when the cytoplasm of hepatocytes collects expansive vacuoles, frequently filled with water, fat, or other substances, as a reaction to cellular

push or damage. This degeneration reflects a disturbance within the ordinary metabolic and detoxification capacities of the liver cells. When vacuolar degeneration gets to be serious, as watched in Bunch G5, it shows that a huge number of hepatocytes are encountering noteworthy brokenness.



**Fig 6:** Histological section of rat liver of G5 showing : (arrows) sever vacuolar degeneration. H&E stain, X400.

#### Conclusion:

The study illustrates that the fluid extricate of grape takes off incorporates a critical affect on cholesterol levels. The discoveries recommend that the extricate can lower cholesterol levels, which may contribute to progressed lipid metabolism and cardiovascular wellbeing. The decrease in cholesterol may well be ascribed to the bioactive compounds display in grape clears out, such as flavonoids, polyphenols, and cancer prevention agents, which have been detailed to play a part in lessening lipid peroxidation and improving cholesterol homeostasis.

By and large, the grape leaf extricate appears promising potential as a characteristic helpful operator for overseeing tall cholesterol and avoiding related wellbeing dangers, such as atherosclerosis and heart infection. Advance investigate is prescribed to investigate the instruments of activity and the long-term impacts of grape leaf extricate on cholesterol and other lipid parameters.

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