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Hepatoprotective Effect of Hydroalcoholic Extract of *Vitis vinifera L* Seeds on Paracetamol-Induced Liver Damage in Wistar Rats.

Navenaa S.G. Pandian, Hemapriya Sankar, Sivakumar Ramalingam*, Renuka Saravanan*

Department of Chemistry and Biosciences, SASTRA Deemed to be University, Srinivasa Ramanujan Centre, Kumbakonam. 612 001, Tamil Nadu, India.

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ABSTRACT

Vitis vinifera L has been used traditionally in Pakistan, Italy, and Turkey as laxatives. Carminatives are used as drug therapy for many diseases. The study aims to determine the hepatoprotective potential of the hydroalcoholic extract *of Vitis vinifera*L (EVV) seeds in paracetamol-induced hepatotoxicity in albino rats. The hepatoprotective effect of the hydroalcoholic extractof *Vitis vinifera* L (EVV) seeds was tested *in vivo* by assessing the antioxidant parameters, liver enzymes, biochemical parameters, and histopathological examinations. Wistar rats-induced hepatotoxicity with paracetamol treated with *Vitis vinifera* L seeds at 200 and 400 mg/kg exhibited decrease in *in vivo* antioxidant parameters, liver enzymes, and biochemical parameters due to the presence of phytoconstituents such asflavonoids, steroids, glycosides, quinine, phenol, and saponin. The histological examinations of liver tissue revealed increased necrosis in untreated animals, whereas the central vein, surrounded by normal cells with good architecture, was observed in EVV-treated animals compared to silymarin-treated groups (25 mg/kg body weight). The scientific findings revealed that the hydroalcoholic extract of *Vitis vinifera* L. seed possesses hepatoprotective potential.

***Keywords*:** Paracetamol, *Vitis vinifera* L seed, Hepatoprotective, Antioxidant.

Introduction

Vitis vinifera, also known as the grape or the black royal cultivar of the Vitaceae family.[1] Grapes come in varieties and species and are grown for several purposes. Earlier studies have shown that grape seeds' inclusion has an anti-inflammatory impact. Other pharmacological effects have been noted, particularly from its seeds, and include neuroprotection, hepatoprotection, wound healing, and anti-seizure action.[2]

The liver is a vital organ that regulates several important biochemical and biological activities like homeostasis, growth, energy, nutrient delivery, drug and other xenobiotics, detoxification, and healing infection. It is highly vulnerable to injury from hepatotoxic substances.[3] An alternated liver function resulting in illness is liver disease or hepatic disease. Many vital bodily processes are carried out by the liver, and if it develops a disease, those processes may cease, which might seriously affect the function of the body.[4]

Liver diseases are quickly becoming acknowledged as a public health priority, according to the WHO (2021). In India, acute liver disease mortality is 5-6.3%, chronic liver disease, including cirrhosis (hepatitis B virus), mortality is 17.6-47.9%, and liver cancer (HBV) mortality is 40-60%.[5]

Paracetamol, an effective pain-relieving and antipyretic drug with fewer side effects at typical therapeutic doses, when taken in overdoses combined with other drugs or alcohol, can cause acute liver damage. As a result, severe central lobular hepatic necrosis, renal failure, and even death in humans and experimental animals can be developed.[6]

*Corresponding author. E mail: renuka@src.sastra.edu; rsiva@src.sastra.edu.
Tel: 91-9944256413; 91-9751545725

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Herbal medicines are safe and devoid of major adverse reactions, which has led to a significant increase in the usage of herbal medicines to treat ailments worldwide. They can also be quickly and readily derived from nature.[7] The current study focuses on the notable hepatoprotective potential of the hydroalcoholic extract of *Vitis vinifera L*. seed through various *in vivo* approaches.

Material and Methods

Seed collection

Vitis vinifera L. seeds, which were collected in and around Kumbakonam, Tamil Nadu, in December 2021 and identified with the aid of a local taxonomist, Dr.N.Ravichandran, Assistant Professor, SRC, SASTRA, Kumbakonam and a voucher specimen number (CARISM00198) was mentioned for further reference.

Extraction of seed materials

The seeds of *Vitis vinifera* L were washed and shade-dried for one day and coarsely powdered with a blender. 200 g of *Vitis vinifera* L seed powder was mixed with 750 mL of hydroalcoholic solvent and kept at room temperature for 48 hrs with occasional shaking. A portion of the extract was used for biochemical analysis, and the remaining was lyophilized.

Preliminary phytochemical screening

Standard textual protocols were used to conduct a preliminary phytochemical screening of *Vitis vinifera L* seed extract.[8]

In-vivo Studies

Experimental animals

Wistar strains of male albino rats weighing 140–170 g were purchased from Biogen Animal Breeders in Hosur. Before the study, the animals were given a five-day adaptation period in a laboratory environment. The animal experimentation was performed as per the guidelines of the Institutional Animal Ethics Committee (CPCSEA Approval No. 790/03/ac/CPCSEA).

Grouping of experimental animals
The animals were divided into 5 groups of 6 rats each, and the experimental design was as follows. Group I served as the normal control; Groups II to V were administered orally with paracetamol (2 g/kg body weight); Groups III and IV were treated with hydroalcoholic extracts of 200 and 400 mg/kg body weight for 15 days, respectively; and Group V served as the positive control; which was treated with silymarin (25 mg/kg).At the end of the experimental period, the rats were sacrificed by cervical decapitation. The blood was drawn, and liver tissue was excised for biochemical analysis. A portion of liver tissue was saved in formalin saline for histological examination, and the remaining tissue was homogenized in 0.1M phosphate buffer, pH 7.4. The liver homogenate was used to determine the antioxidant activity.

Determination of body weight
The basic tabletop balance was used to determine the body weight of the experimental rats. After the study period, the animals' body weights were measured before and after the administration of paracetamol.
Determination of blood parameters

Estimation of Liver enzymes
The activity of serum aspartate transaminase, alanine transaminase, and alkaline phosphatase was determined, as described by King (1965).[9] AST catalyzes the conversion of aspartate into ketoglutarate. The formed oxaloacetate was measured by reaction with 2,4 dinitrophenyl hydrazine and can be measured at 520 nm. ALT can be estimated by the formation of pyruvate with DNPH and measured colorimetrically at 520 nm.[9] The activity of ALP was determined by the liberation of phenol. The color developed was read calorimetrically at 640 nm.[9] The activity of serum gamma-glutamyltransferase was measured using the method of Rosalki and Rau (1972).[10] Gamma-glutamyl transferase catalyzes the conversion of a colorless substrate into a colored product, p-nitroaniline. The change in absorbance is directly proportional to the activity of GGT, which is measured at 410 nm. The GGT activity was given as a unit/liter.[10]

Blood glucose estimation
Blood glucose was estimated by the method of Folin O. and Wu H. Cupric ions are converted to cuprous ions when glucose is heated with an alkaline copper solution. To test the cuprous ion colorimetrically, phosphomolybdic acid is added, which is converted to molybdenum blue measured at 620 nm.[11]

Estimation of Serum Bilirubin
Using Malloy and Evelyn's (1937) procedure[12], the serum bilirubin concentration was determined. While bilirubin, coupled with albumin must first be hydrolyzed, bilirubin glucuronate is soluble in water and reacts immediately. The amount of produced azobilirubin color is related to the sample's overall bilirubin concentration.[12]

Lipid Estimation
Cholesterol was determined by the method of Zak[13], triglyceride[14], and high-density lipoprotein cholesterol.[15] The concentration of very low-density lipoprotein cholesterol and low-density lipoprotein cholesterol was determined by Friedewald*et al* (1972).[15]

$$\text{LDL Cholesterol} = \frac{(\text{TC}) - (\text{TGL}) - (\text{HDL Cholesterol})}{5}$$

$$\text{VLDL Cholesterol} = \frac{(\text{TGL})}{5}$$

Estimation of Total Protein
Serum protein concentration was determined by the method of Lowry.[16]Alkaline Copper sulfate solution catalyzes the oxidation of aromatic amino acids followed by the reduction of phosphomolybdic and phosphotungstic acid, resulting in a purple color complex whose intensity is proportional to the quantity of aromatic amino acid, which is measured at 660 nm.

Antioxidant studies
The levels of lipid peroxidation,[17] reduced glutathione,[18] activity of superoxide dismutase[19] and catalase[20] were determined in the liver homogenate of experimental animals.
Histopathological studies
For histological analyses, a small portion of the liver was excised from each group,and stored in neutral formalin saline. In the laboratory, the tissues were mounted using paraffin slices ranging from 5 to 10 mm. The sections were then stained with eosin and hematoxylin dye.[21] R. Thirupurasundari, MD, a pathologist in Kumbakonam, investigated the extent of liver damage.

Statistical analysis
A student's "t" test was used for the data analysis, and the values are expressed as mean ± SE. To compare the significant differences between the groups, a one-way ANOVA was performed. $P<0.05$ was used to determine the significant values.

Results and Discussion

The grape (*Vitis vinifera*), one of the world's largest fruit harvests and the most consumed worldwide, is a food high in antioxidants. The main phenolic antioxidants in grape seed extracts are investigated for their possible hepatoprotective effects.[22]
Table 1 represents the phytochemical screening in dry powder and hydroalcoholic extract of *Vitis vinifera* L. seeds. Both the dry powder and hydroalcoholic extract were found to contain flavones, steroids, glycosides, quinone, and phenol. Terpenoids and coumarin were present only in dry powder, whereas hydroalcoholic extract contains saponin. Anthraquinones, alkaloids, and tannins were absent in both dry and hydroalcoholic extracts of *Vitis vinifera* seed. Flavones are structural derivatives with conjugated aromatic systems; they are typically combined with sugars as glycosides and are naturally phenolic and water-soluble. They act as antioxidants, therefore guarding against degenerative illness. 23 Tannins are a type of plant polyphenol with a bitter taste that binds to proteins and causes them to degrade or shrink. By scavenging free radicals, chelating transition metals, blocking prooxidative enzymes, and preventing lipid peroxidation, they serve a physiological purpose as antioxidants,[23] thereby modulating oxidative stress and preventing degenerative disease.
In Table 2, the experimental animals treated with paracetamol showed a significant decrease in body weight, whereas the groups treated with EVV showed a significant increase in body weight, which was comparable to the group treated with silymarin (25 mg/kg b.w.). The loss of weight was brought on by the direct toxicity of paracetamol and/or indirect toxicity related to liver injury.

Table 1: Phytochemical screening in dry powder and hydro-alcoholic extract of *Vitis vinifera* seed

Phytoconstituents	Dry Powder	Hydroalcoholic extract
Flavones	+	+
Steroids	+	+
Terpenoids	+	-
Anthraquinones	-	-
Glycosides	+	+
Alkaloids	-	-
Quinones	+	+
Phenols	+	+
Tannins	-	-
Saponin	-	+
Coumarin	+	-

+ : Presence - : Absence

Rats administered with paracetamol, group II, at 200 mg/kg.b.w. had significantly lower body weight than the control, EVV-treated groups,

and standard medication treatment groups, which showed a similar report conducted by Mohd Mujahid et al. (2017).[24]

Table 3 showed an increase in liver marker enzyme activity in group II animals induced by paracetamol of about 140.45 U/L, 94.5 U/L, 82.54 IU/L, and 258.5 IU/L, whereas the group treated with *Vitis vinifera* seed extract showed a significant decrease in dose-dependent manner of about 83.10 U/L,40.5 U/L, 52.73 IU/L and 130.5 IU/L respectively, compared to group II. The group treated with silymarin also showed a significant decrease in levels, which is compared against the paracetamol-induced group.

The Enzyme markers such as AST, ALT, GGT, and ALP are predominantly found in the mitochondria of hepatocytes and are more specific to the liver for detecting liver injury. These enzyme markers are the most common biochemical markers to evaluate liver injury. The increase in enzyme levels is a clear sign of cellular leakage and a loss of the cell membrane's functional integrity.[25]

The results showed an increase in blood glucose and total bilirubin levels in Group II compared to normal control. The group was treated with a dose of *Vitis vinifera* seed extract of dose 200 mg/kg b.w. and 400 mg/kg b.w. showed significant a decrease in level which is compared to Group II (negative control). The silymarin-treated group also showed a significant decrease compared with the paracetamol-induced group.[26] Following paracetamol intoxication, the total bilirubin and glucose levels increased, as seen in Table 4. Intoxicated rats given paracetamol had their direct and indirect bilirubin levels reduced after receiving seed extract. Mohd Mujeeba *et al* (2011) also showed similar data in the study.[26]

The level of cholesterol and triglyceride increased on paracetamol induction compared to group I (Table 5). The experimental animals that received *Vitis vinifera* seeds extract at different concentrations showed a significant decrease in cholesterol and triglyceride levels compared to group II. According to this finding, liver damage caused the disease-control animals' lipid content to increase, and treatment with a hydro-alcoholic extract of *Vitis vinifera L* seeds led to a good recovery in a dose-dependent way. Due to the liver cells' inability to handle the lipids, it has been demonstrated in numerous prior research that the animals with damaged livers had higher cholesterol and triglyceride levels.[27]

As indicated in Table 4, intoxicated rats showed a significant decrease in high-density lipoproteins, whereas low-density lipoproteins and very low-density lipoproteins showed a significant increase compared to Group I. On treatment with *Vitis vinifera* extract, altered values changed significantly compared to Group II. The group that received silymarin (25 mg/kg b.w.) also showed significant changes compared with the paracetamol-administered group. The primary cellular mechanisms implicated in the development of fatty liver brought on by paracetamol are radical generation and lipid peroxidation. Large-scale lipid buildup is thought to be a harmful situation, and when it persists for an extended period, the cells undergo fibrotic alterations that lead to cirrhosis and reduced liver function. The production of fatty acids and triglycerides from acetate is accelerated by paracetamol. This might be because more substrate (acetate) is available as a result of acetate being transported into the liver cell. The production of cholesterol is also elevated in paracetamol intoxication.[28] Furthermore, the liver is crucial to the metabolism of lipids. A significant increase in LDL,VLDL and decrease in HDLlevel was observed in Group II rats as compared to Group I rats (Table 6). According to Mahmoodzadeh*et al* (2017), paracetamol increases cholesterol synthesis and accelerates the transfer of acetate into liver cells (perhaps by improving the availability of acetate). Additionally, it improves lipid esterification and boosts the production of fatty acids and triglycerides from acetate. Triglyceride buildup in the liver may result from decreased lysosomal lipase activity and VLDL production.[29]

Table 7 indicates that paracetamol caused a significant decrease in total protein level from 8.80±0.01 to 5.75±0.53 gm after intoxication. However, Group III and IV, treated with EVV, showed an increase in total protein to 7.74±0.20 and 8.81±0.19 gm, respectively ($P<0.05$).

According to recent studies, paracetamol-induced hepatotoxicity caused the levels of total protein, albumin, and globulin to decrease. The drop is linked to the initial injury that occurs and is localized in the endoplasmic reticulum and culminates in P450 loss, functional failure, a decrease in protein synthesis, and an accumulation of triglycerides that causes fatty liver.[30]

Table 8 showed a significant change in antioxidant activity in paracetamol-induced rats. A significant increase in superoxide dismutase activity, a decrease in catalase activity, and a reduced glutathione level in Group II were observed compared to Group I. The groups treated with *Vitis vinifera* seed extract showed significant changes in the lipid peroxide level, reduced glutathione,and the activity of catalase when compared to Group II animals. Group V also showed a significant change compared to Group II paracetamol-induced hepatotoxic animals.

Table 2: Effect of Hydroalcoholic *Vitis vinifera L* seeds extract on experimental Hepatotoxic rat's body weight

Groups	Body Weight(g)		
	1st Day	7th Day	14th Day
Group I	120 ± 0.37	150 ± 1.83	180 ± 1.83
Group II	120 ± 0.73*	110 ± 1.83*	100 ± 1.83*
Group III	120 ± 1.83**	120 ± 3.65**	150 ± 3.65**
Group IV	120 ± 1.83**	150 ± 3.65**	170 ± 1.83**
Group V	120 ± 0.73**	140 ± 3.65**	170 ± 3.65**

Values are Mean ± SE, n = 5;* $p<0.05$ statistically significant when compared with Group I;**$p<0.05$ Statistically significant when compared to Group II

Group I represents normal control; Group II represents negative control; Group III and IV represent *Vitis vinifera* seeds extract of 200 mg and 400 mg; Group V represents Positive control.

Table 3: Effect of Hydroalcoholic Extract of *Vitis vinifera* seed on liver enzymes makers on paracetamol induced Wistar Rats

Groups	AST (U/L)	ALT (U/L)	GGT(IU/L)	ALP(IU/L)
Group I	58.60 ± 1.39	35.6 ± 0.8	49.24 ± 0.16*	98.6 ± 0.80*
Group II	140.45 ± 2.46*	94.5 ± 0.8*	82.54 ± 1.12	258.5 ± 0.88
Group III	112.69 ± 1.40**	55.9 ± 1.6**	60.49 ± 1.50	194.9 ± 1.64
Group IV	83.10 ± 01.60**	40.5 ± 1.0**	52.73 ± 0.98**	130.5 ± 1.06**
Group V	78.15 ± 0.54**	38.6 ± 0.8**	50.40 ± 0.84**	114.6 ± 0.86**

Values are Mean ± SE, n = 5; * $p<0.05$ statistically significant as compared with Group I;
**$p<0.05$ Statistically significant as compared to Group II

Group I represents normal control; Group II represents negative control; Group III and IV represent *Vitis vinifera* seed extract of 200 mg and 400 mg; Group V represents Positive control.

One symptom of oxidative stress is lipid peroxidation. Increased MDA levels in the liver caused by paracetamol suggested hyperlipid peroxidation, which damaged hepatic tissue and rendered the antioxidant defense mechanisms ineffective in avoiding the generation of excess free radicals.[31]According to Mansourian, the prevention of paracetamol-induced hepatopathy depends on antioxidant activity or the reduction of free radical production.[32] The animal system has a strong defense system that can stop and reverse harm brought on by free radicals. This is attained by endogenous antioxidant enzymes like glutathione peroxidase, superoxide dismutase, and catalase. These enzymes work together as a cohesive team to combat reactive oxygen species.[33]

Table 4: Effect of Hydroalcoholic extract of *Vitis vinifera* seed on Blood glucose and Total Bilirubin on Paracetamol-induced Wistar Albino Rats

Groups	Blood Glucose (mg/dL)	Total Bilirubin (mg/dL)
Group I	103.45 ± 4.34	0.73 ± 0.09
Group II	279.23 ± 0.95*	3.2 ± 0.66*
Group III	106.57 ± 2.49**	1.03 ± 0.13**
Group IV	73.24 ± 2.14**	1.00 ± 0.11**
Group V	118.48 ± 1.39**	1.03 ± 0.13**

Values are Mean ± SE, n = 5;
* p<0.05 statistically significant as compared with Group I
**p<0.05 Statistically significant as compared to Group II
Group I represents normal control; Group II represents negative control; Group III and IV represent *Vitis vinifera* seed extract of 200 mg and 400 mg; Group V represents Positive control.

Table 5: Effect of Hydroalcoholic Extract of *Vitis vinifera L* seedon Cholesterol and Triglycerides of Experimental Animals

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Group I	166.04 ± 1.69	184.25 ± 2.23
Group II	216.07 ± 1.52*	210.59 ± 1.93*
Group III	203.87 ± 1.39**	198.98 ± 2.91**
Group IV	179.17 ± 0.74**	189.99 ± 2.44**
Group V	170.72 ± 0.59**	186.12 ± 2.22**

Values are Mean ± SE, n = 5;
* p<0.05 statistically significant as compared with Group I;
**p<0.05 Statistically significant as compared to Group II
Group I represents normal control; Group II represents negative control; Group III and IV represent *Vitis vinifera* seed extract of 200 mg and 400 mg; Group V represents Positive control.

Table 6: Effect of Hydroalcoholic Extract of *Vitis vinifera* seed on HDL, LDL, and VLDL of Experimental animal.

Groups	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group I	42.93 ± 2.19	15.41 ± 0.74	12..84 ± 0.44
Group II	25.70 ± 0.73*	92.19 ± 0.64*	32.31 ± 0.38*
Group III	32.94 ± 0.96**	55.32 ± 0.97**	18.19 ± 0.58**
Group IV	39.52 ± 1.40**	33.32 ± 0.81**	15.99 ± 0.49**
Group V	40.72 ± 2.26**	24.37 ± 0.74**	14.82 ± 0.44**

Values are Mean ± SE, n = 5;
* p<0.05 statistically significant as compared with Group I;
**p<0.05 Statistically significant as compared to Group II
Group I represents normal control; Group II represents negative control; Group III and IV represent *Vitis vinifera* seed extract of 200 mg and 400 mg; Group V represents Positive control.

Table 7: Effectof Hydroalcoholic Extract of*Vitisvinifera L*Seeds on Total Protein, Albumin and Globulin in Experimental Animal.

Groups	TotalProtein (gm)	Albumin(gm)	Globulin(gm)
GroupI	8.80 ± 0.01*	5.77 ± 0.06*	2.97 ± 0.02*
Group II	5.75 ± 0.53	3.54 ± 0.05	2.55 ± 0.02
Group III	7.74 ± 0.20	5.54 ± 0.27	2.54 ± 0.01
GroupIV	8.81 ± 0.19**	6.29 ± 0.28**	2.89 ± 0.029**
GroupV	8.67 ± 0.04**	5.70 ± 0.02**	2.97 ± 0.022**

Values areMean ±SE,n=5;
*p<0.05statistically significant as comparedwithGroup II;
**p<0.05statistically significant as compared with group II;
Group I represents normal control; Group II represents negative control; Group III and IV represent *Vitis vinifera* seed extract of 200 mg and 400 mg; Group V represents Positive control.

Due to the substantial free radical production that damages hepatic tissues in the current investigation, the endogenous enzymatic and non-enzymatic antioxidants were restored after treatment with the study medication.[34]

Histological studies of the liver

Figure 1 represents the effect of hydroalcoholic extract of *Vitis vinifera* seed on histopathological examination of liver tissue of paracetamol-induced hepatotoxic animals. (A) Microscopic examination of Group I, where hepatocytes were seen in a normal liver in the histological section of rat liver stained with hematoxylin and eosin. (B) Microscopic examination of paracetamol-induced rats (Group II), where hepatocytes with patchy necrosis are visible in the histological section of rat liver stained with hematoxylin and eosin was observed. (C) Microscopic examination of paracetamol-induced and treated with *Vitis vinifera* seed extract (200 mg/kg b.w.) shows the central vein is surrounded by patchy hepatocyte necrosis in the histological section of rat liver stained with hematoxylin and eosin. (D) Microscopic examination of paracetamol-induced and treated with *Vitis vinifera* seed extract (400 mg/kg b.w.) reported the absence of hepatocyte necrosis. (E) Microscopic examination of paracetamol-induced and treated with silymarin exhibits considerable periportal lymphocytic infiltration and no necrosis in the histological slice stained with hematoxylin and eosin.

The hepatoprotective effect of *Vitis vinifera* seed was further confirmed by the histological examinations of the liver, where necrosis was seen in the paracetamol-induced group, and the group treated with extract showed a reversal of damaged tissue to near normal. This was in accordance with Zuzana Papackova*et al,* (2018), where inflammation progressed in the acetaminophen-induced hepatotoxic group but diminished after treatment.[35]Necrosis appears to be the dominant cell death pathway in APAP intoxication, which is partially preventable with drug treatment.[3]

Conclusion

In the present study, oral administration of hydroalcoholic extract of *Vitis vinifera* Lseeds significantly altered the antioxidant parameters, liver enzymes, biochemical parameters, and, histopathological studies against paracetamol-induced hepatotoxicity in Wistar rats. It was evident that *Vitis vinifera* L seeds have hepatoprotective potential to treat liver diseases.

Conflict of Interest

The authors declare no conflict of interest.

Table 8: Effect of Hydroalcoholic extract of *Vitis vinifera L.*seeds on Antioxidant enzymes and the level of LPO and GSH in experimental animals

Groups	LPO (nmol MDA/g tissue)	SOD (mM of epinephrine oxidized/min/mg of protein)	Reduced Glutathione (mg/gm tissue)	Catalase (mM of H_2O_2 oxidized / min/mg of protein)
Group I	24.24 ± 0.02	12.64 ± 0.54	28.69 ± 0.16	52.24 ± 0.14
Group II	158.47 ± 0.11*	217.92 ± 2.86*	9.47 ± 0.18*	21.49 ± 0.61*
Group III	104.78 ± 0.55**	109.83 ± 1.81**	15.99 ± 0.02**	30.26 ± 0.51**
Group IV	52.64 ± 0.05**	48.33 ± 0.52**	21.67 ± 0.03**	44.14 ± 1.72**
Group V	67.24 ± 0.07**	67.24 ± 0.34**	23.09 ± 0.18**	.22 ± 0.48**

Values are Mean ± SE, n = 5;
* p<0.05 statistically significant when compared with Group I;
**P<0.05 Statistically significant when compared to Group II
Group I represents normal control; Group II represents negative control; Group III and IV represent *Vitis vinifera* seed extract of 200 mg and 400 mg; Group V represents Positive control.

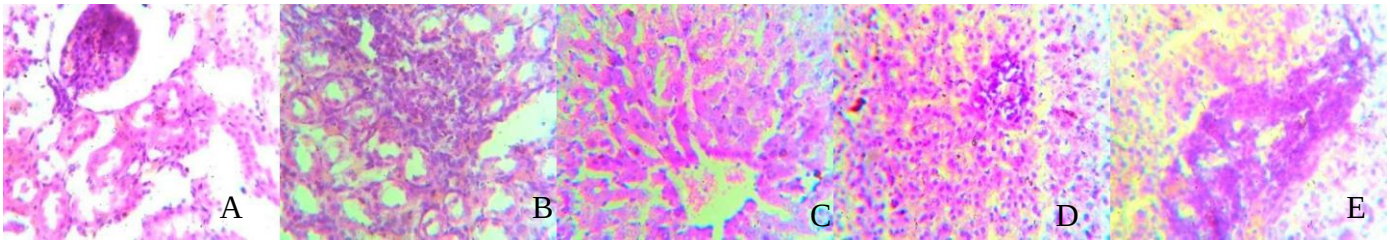

Figure 1: Histopathological images of Wistar rat's Liver tissues of (A) Normal control; (B) negative control (2g paracetamol /kg b.w); (C) treatment with *Vitis vinifera* L. extract (200 mg/kg b.w); (D) treatment with *Vitis vinifera* L. extract (400 mg/ kg b.w); (E) positive control (25 mg silymarin/kg b.w.).

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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