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# The bioactive molecule resveratrol (RVTL) obtained from the black grapes (*Vitis vinefera*) act as potential hepatocytes regenerators and cytotoxic agent

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## ABSTRACT

*The main aim and objective of the present research work was the extraction, isolation and characterization of bioactive molecule resveratrol (RVTL) from the black grapes (Vitis vinefera) and in vivo evaluation of hepatocytes regenerator potentiality against $CCl_4$ induced rat hepatocytes and in vitro cytotoxic activity against both human colon cancer cell line HT-29 and human breast cancer HS 578T Cell line. Based on this a new series of constituents had been planned to extract by Ethanol (ENL) from black grapes. The isolation and characterization of bioactive molecule resveratrol (RVTL) were carried out from the ENL extract of the black grapes (Vitis vinefera) by modern analytical techniques such as U.V, I.R, $^{1}$H-NMR and $^{13}$C-NMR, JEOL GCmate and HPLC–ESI–MS/MS spectroscopy. In-vivo hepatoprotective activity was carried out by using albino rats. The results displayed that the elevated levels of SGOT, SGPT, ALP and Serum bilirubin were mainly due to $CCl_4$ intoxication, reduced significantly (*P<0.05) in rats, after treatment with ENL extract containing RVTL of black grapes (Vitis vinefera). Treatment with ENL extract of black grapes (Vitis vinefera) at a both doses of 250 and 500 mg/kg b.w. significantly decreased the SGOT, SGPT, ALP, Serum Bilirubin levels by 23.5%, 28.9%, 8.9%, and 17.2% (at low dose) and 26.9%, 35.9%, 14.5% and 22.3% (at high dose) respectively. Silymarin used as standard drug showed a reduction of 56.09%, 69.89%, 57.46% and 19.04%$^{ns}$ receiving $CCl_4$ alone. Histopathological investigation displayed that at both doses (250 mg/kg b.w. and 500 mg/kg b.w.), the ENL extract of black grapes (Vitis vinefera) was possessed very good hepatoprotective activity, but at 500 mg/kg b.w. executed excellent hepatoprotective activity against CCl4 induced damaged hepatocytes. The in vitro cytotoxic activity was carried out by SRB assay. The results obtained from the in-vitro studies performed by SRB assay using the HT-29 cell lines and HS 578T displayed that the ENL extract containing RVTL of black grapes (Vitis vinifera) possessed a very good cytotoxic activity. From the present studied it had been concluded that ENL extract containing RVTL of black grapes (Vitis vinifera) exhibiting the potential capability to kill the neoplastic cell when compared with standard drug 5-FU and doxorubicin. The ENL extract containing RVTL of black grapes (Vitis vinifera) displayed with the highest 93.43% growth inhibition at 12.5 µg ($IC_{50}$ = 2.2 µg/ml) against human colon cancer cell line HT-29 and 93.6% growth inhibition at 25 µg ($IC_{50}$ = 1.9 µg/ml) against human breast cancer HS 578T Cell line.*

**Key words:** Hepatoprotective, HT-29, HS 578T, Bioactive molecule, SRB, IC50 etc.

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## INTRODUCTION

**Resveratrol** (3,5,4'-trihydroxy-*trans*-stilbene) is a stilbenoid, a type of natural phenol, and a phytoalexin produced naturally by several plants in response to injury or when the plant is under attack by pathogens such as bacteria or fungi [1]. Food sources of resveratrol include the skin of grapes, blueberries, raspberries, and mulberries [2]. As of 2014, there is limited evidence of health effects in humans.

**Occurrences**

**Plants:** Resveratrol was originally isolated by Takaoka from the roots of hellebore in 1940, and later, in 1963, from the roots of Japanese knotweed. It attracted wider attention only in 1992, however, when its presence in wine was suggested as the explanation for cardioprotective effects of wine [3].

In grapes, trans-resveratrol is a phytoalexin produced against the growth of fungal pathogens such as Botrytis cinerea [4]. Its presence in Vitis vinifera grapes can also be constitutive, with accumulation in ripe berries of different levels of bound and free resveratrols, according to the genotype [5]. In grapes, resveratrol is found primarily in the skin [6] and, in muscadine grapes, also in the seeds [7] The amount found in grape skins also varies with the grape cultivar, its geographic origin, and exposure to fungal infection. The amount of fermentation time a wine spends in contact with grape skins is an important determinant of its resveratrol content [6].

**Foods:** The levels of resveratrol found in food varies greatly. Red wine contains between 0.2 and 5.8 mg/l,[8] depending on the grape variety, while white wine has much less, because red wine is fermented with the skins, allowing the wine to extract the resveratrol, whereas white wine is fermented after the skin has been removed [6][9]. The composition of wine is different from that of grapes since the extraction of resveratrols from grapes depends on the duration of the skin contact, and the resveratrol 3-glucosides are in part hydrolysed, yielding both trans- and cis-resveratrol [10]. A number of reports have indicated muscadine grapes may contain high concentrations of resveratrol, and that wines produced from these grapes, both red and white, may contain more than 40 mg/l, [7], [11] however, subsequent studies have found little or no resveratrol in different varieties of muscadine grapes [12], [13].

One of the most promising sources is peanuts, especially sprouted peanuts where the content rivals that in grapes. Before sprouting, it was in the range of 2.3 to 4.5µg/g, and after sprouting, in the range of 11.7 to 25.7 µg/g depending upon peanut cultivar [14]. The fruit of the mulberry (esp. the skin) [15] is a source, and is sold as a nutritional supplement.Cocoa powder, baking chocolate, and dark chocolate also have low levels of resveratrol in normal consumption quantities (0.35 to 1.85 mg/kg) [16].

**Pharmacological activities**

There are a number of promising animal studies and some data from human clinical trials is emerging [17],[18] Nevertheless, there is not enough evidence to recommend consumption of resveratrol beyond the amount that can be obtained through dietary sources, and more human clinical trials are needed [19].

**Cancer:** As of 2014, the results of limited human clinical trials with small samples sizes of the effects of resveratrol on cancer are inconsistent. Testing of resveratrol in animal models of cancer have also shown mixed results [20]. The strongest evidence of anticancer action of resveratrol exists for tumors it can contact directly, such as skin and gastrointestinal tract tumors. For other cancers, the evidence is uncertain, even if massive doses of resveratrol are used [21]. Resveratrol treatment appeared to prevent the development of mammary tumors in animal models; however, it had no effect on the growth of existing tumors. Paradoxically, treatment of prepubertal mice with high doses of resveratrol enhanced formation of tumors. Injected in high doses into mice, resveratrol slowed the growth of neuroblastomas [21].

**Cardioprotective effects:** Moderate drinking of red wine has long been known to reduce the risk of heart disease [22] This is best known as "the French paradox" [23], [24]**.** Studies suggest resveratrol in red wine may play an important role in this phenomenon [25]. It appears to stimulate endothelial nitric oxide synthase (eNOS) activity; [26] and inhibition of platelet aggregation [27]. The cardioprotective effects of resveratrol also are theorized to be a form of preconditioning the best method of cardioprotection, rather than direct therapy [28] Study into the cardioprotective effects of resveratrol is based on the research of Dipak K. Das. However, he has been found guilty of scientific fraud, and many of his publications related to resveratrol have been retracted [29] [30].

**Antidiabetic effects:** Other diabetic animal model studies by different researchers have also demonstrated the antidiabetic effects of resveratrol [31], [32] This compound was shown to act as agonist of PPARgamma, nuclear receptor that is current pharmacological target for the treatment of diabetes type 2 [33]

**Skin protection:** The oxidative stress induced by ultraviolet radiation is one of the main causes for premature skin ageing. The photoprotective effects of several polyphenols known for their antioxidant properties, including resveratrol, have been investigated in silico and in topical application conditions.[34], [35]

**Neuroprotective effects:** The neuroprotective effects have been confirmed in several animal model studies [36]

**Sirtuin activation:** Some of the benefits demonstrated in previous studies were overstated [37], [38] however, this study was challenged immediately, [39] and a few experiments were suggested to be of inferior quality [40].

**Psychological:** In a number of animal models resveratrol has had an antidepressant-like effect. Whether or not there is any effect in humans is unclear [41].

## MATERIALS AND METHODS

### Drug and chemicals

The standard drugs Silymarin (hepatoprotective agent), 5-flurouracil and Doxorubicin (anticancer drugs) purchased from Local Retail Pharmacy Shop and solvents and other chemicals were used for the extraction from Institutional Store and were of AR grade. In the present study the ***in-vivo*** hepatoprotective activity was evaluated by $CCl_4$ induced hepatotoxicity model in rats.

### Instrumentation

The IR spectra were recorded in the solid state as a KBr dispersion medium using the FT-IR (Perkin Elmer, Spectrum 65 & JASCO-FT-IR-430) spectrophotometer. The UV spectrum was recorded on a Shimadzu UV-visible spectrophotometer using acetonitrile as the medium. The 1H NMR and 13C NMR experiments for resveratrol were performed at 400.13 MHz and 100.62 MHz, respectively, on the Bruker Avance 400 MHz FT NMR spectrometer with a multinuclear BBO probe. DMSO- d6 was used as the solvent. HPLC/MS/MS analyses were performed using a system consisting of a Finnigan autosampler a Finnigan LC pump, a Finnigan TSQ Quantum Ultra equipped with an electrospray ion source and operated by XCalibur software. HPLC–ESI–MS/MS and was adopted to analyze trans-resveratrol. The Mass spectra of trans-resveratrol also recorded by JEOL GCmate.

### Experimental animals

White male albino rats weighing about 200-250 g were used. They were obtained from the animal house of C.L. Baid Metha College of Pharmacy, Chennai. They were kept under observation for about 7 days before the onset of the experiment to exclude any intercurrent infection, had free access to normal diet and water. The animals were housed in plastic well aerated cages at normal atmospheric temperature (25±5 °C) and normal 12- hour light/dark cycle under hygienic conditions.

### Cell cultures

The cell culture human colon cancer cell line **HT-29** and human breast cancer **HS 578T Cell line** were provided by National Centre for Cell Science (NCCS), Pune and were grown in Eagles Minimum Essential Medium (EMEM) which contained 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 100% relative humidity, 5% CO2, 95% air and the culture medium was changed twice a week.

### Methodology for extraction [42]

Weigh 20 g of black grapes paste (ripen can be mashed to prepare a paste) into a 250 ml round-bottomed flask. Add 50 ml of ethanol and 60 ml of dichloromethane. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separatory funnel. Wash this mixture containing bioactive compounds with three portions of 150 ml each with sodium chloride solution. Dry the organic layer over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating.

### Preliminary Phytochemical screening [43, 44, 45]

Preliminary Phytochemical screening of ENL extract of black grapes (***Vitis vinifera***) had shown the presence of various bioactive compounds such as carbohydrates, aminoacids and peptides, phytosterols, carotenoids and polyphenols (higher concentration).

**Characterization of Resveratrol (RVTL)**

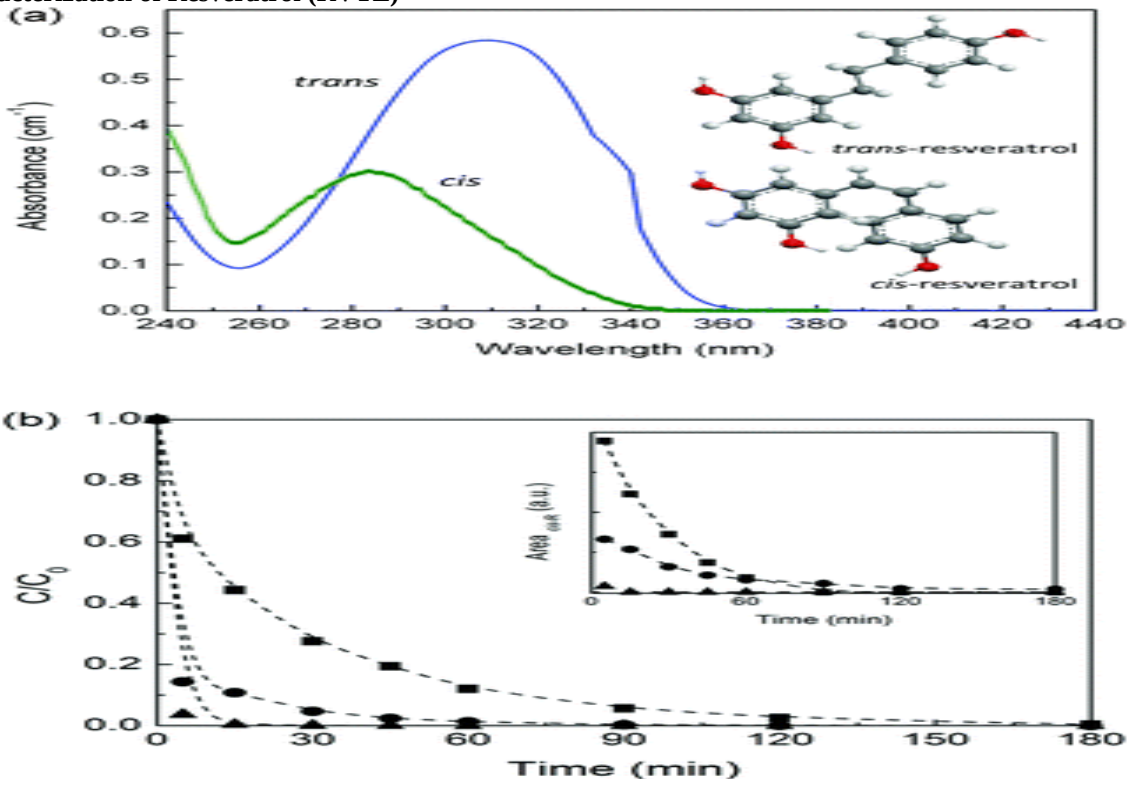



**Fig 1: U.V spectra for trans and cis- Resveratrol**

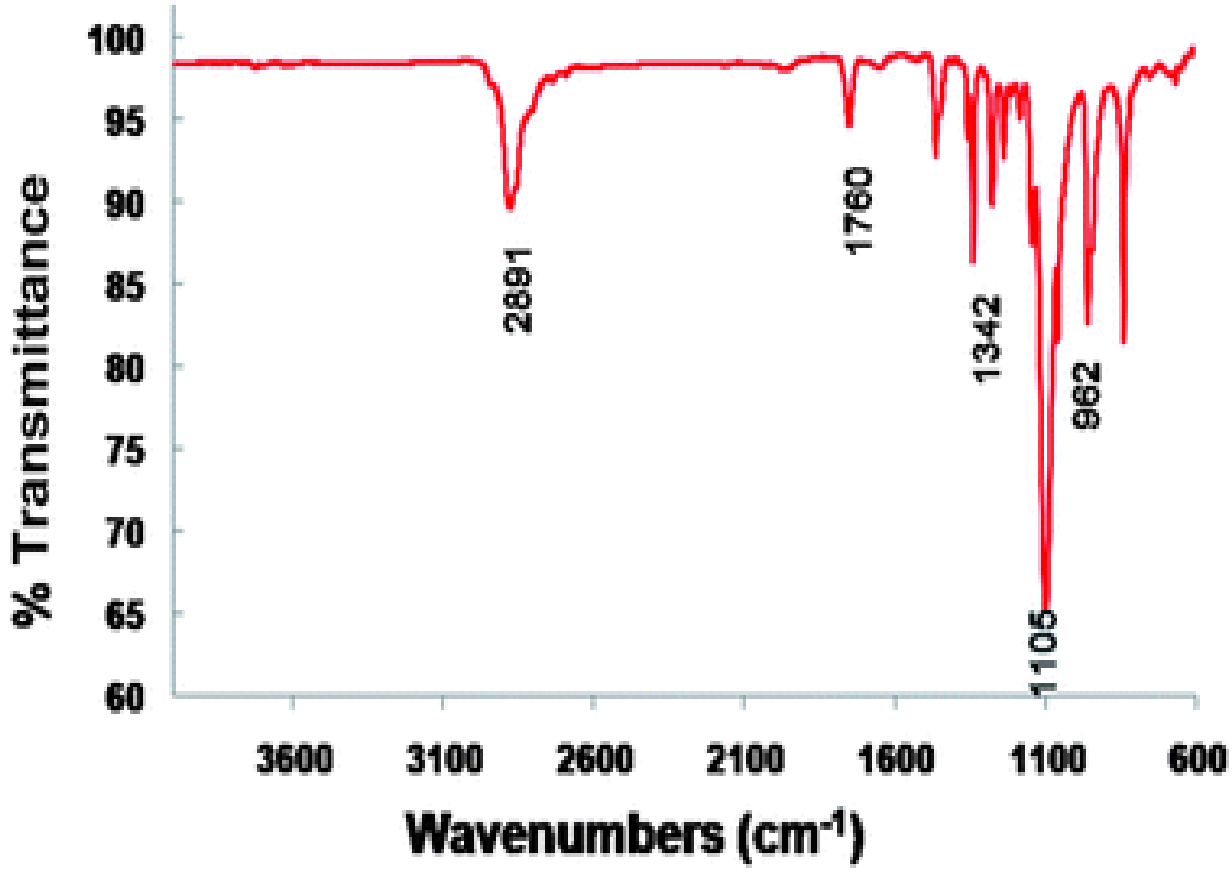



**Fig 2a: IR spectra for Resveratrol**

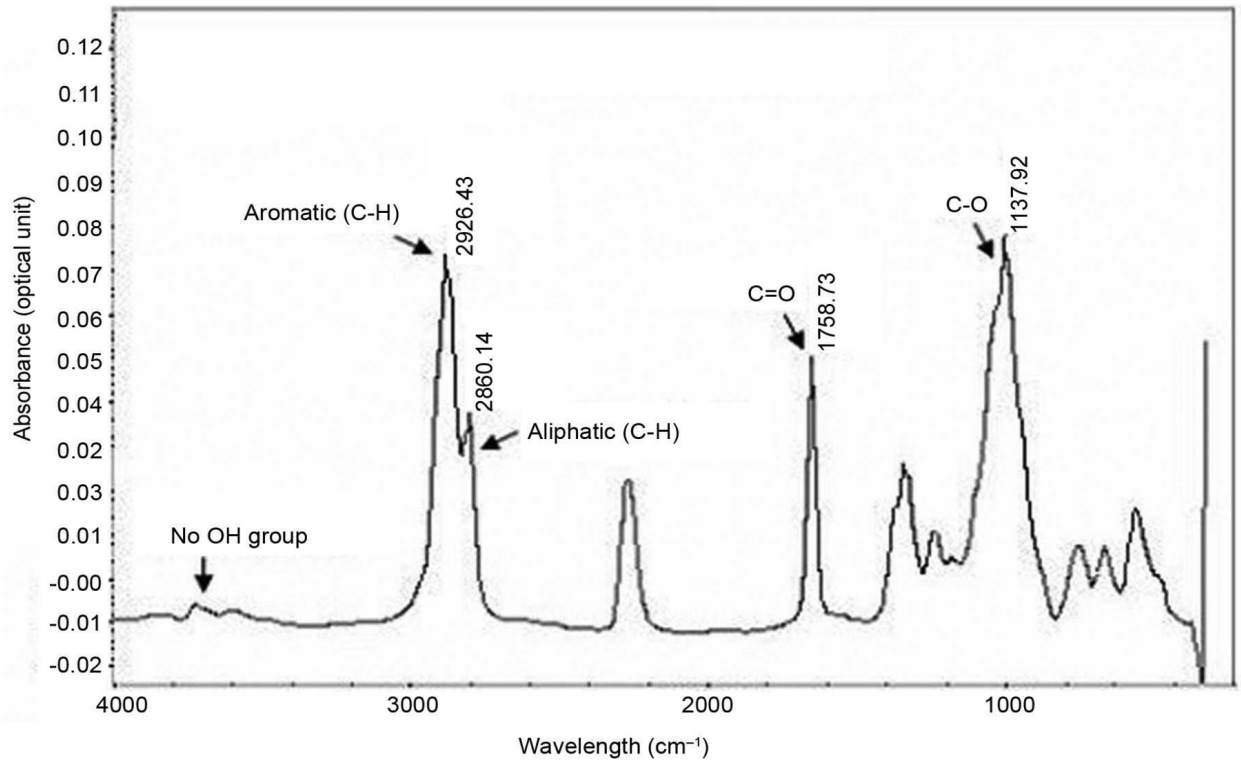



**Fig 2b: IR spectra for Resveratrol**

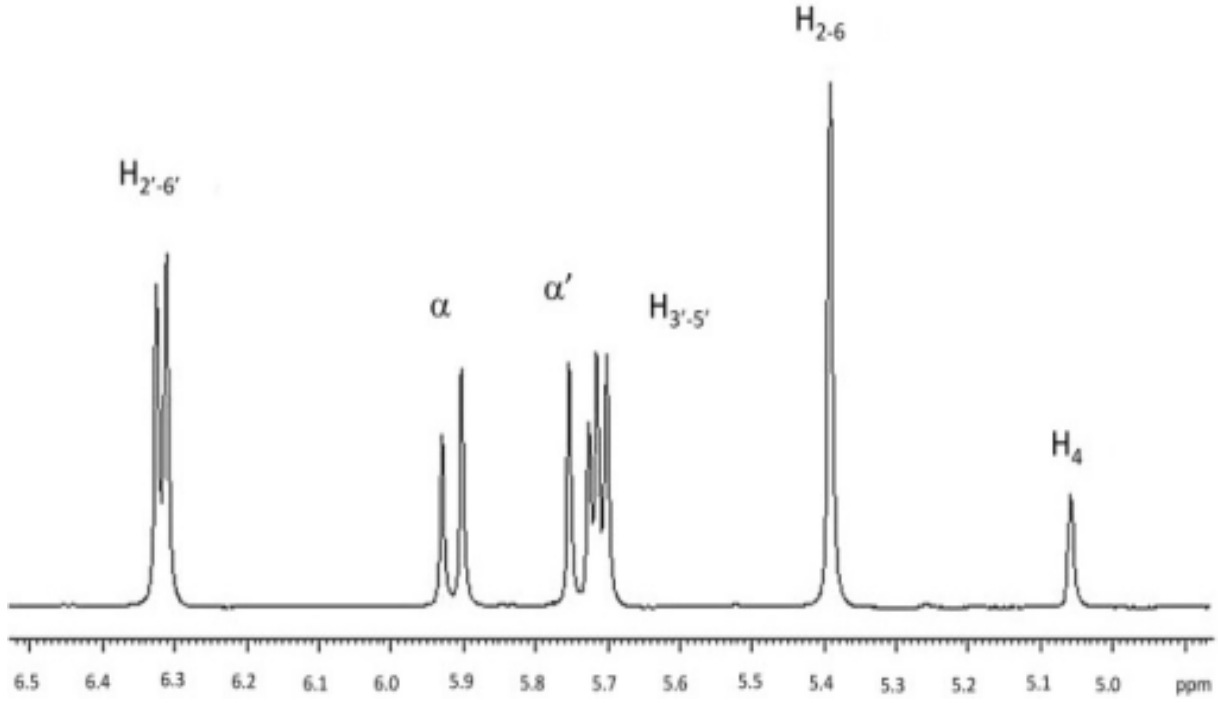



**Fig 3a: NMR spectra for Resveratrol**

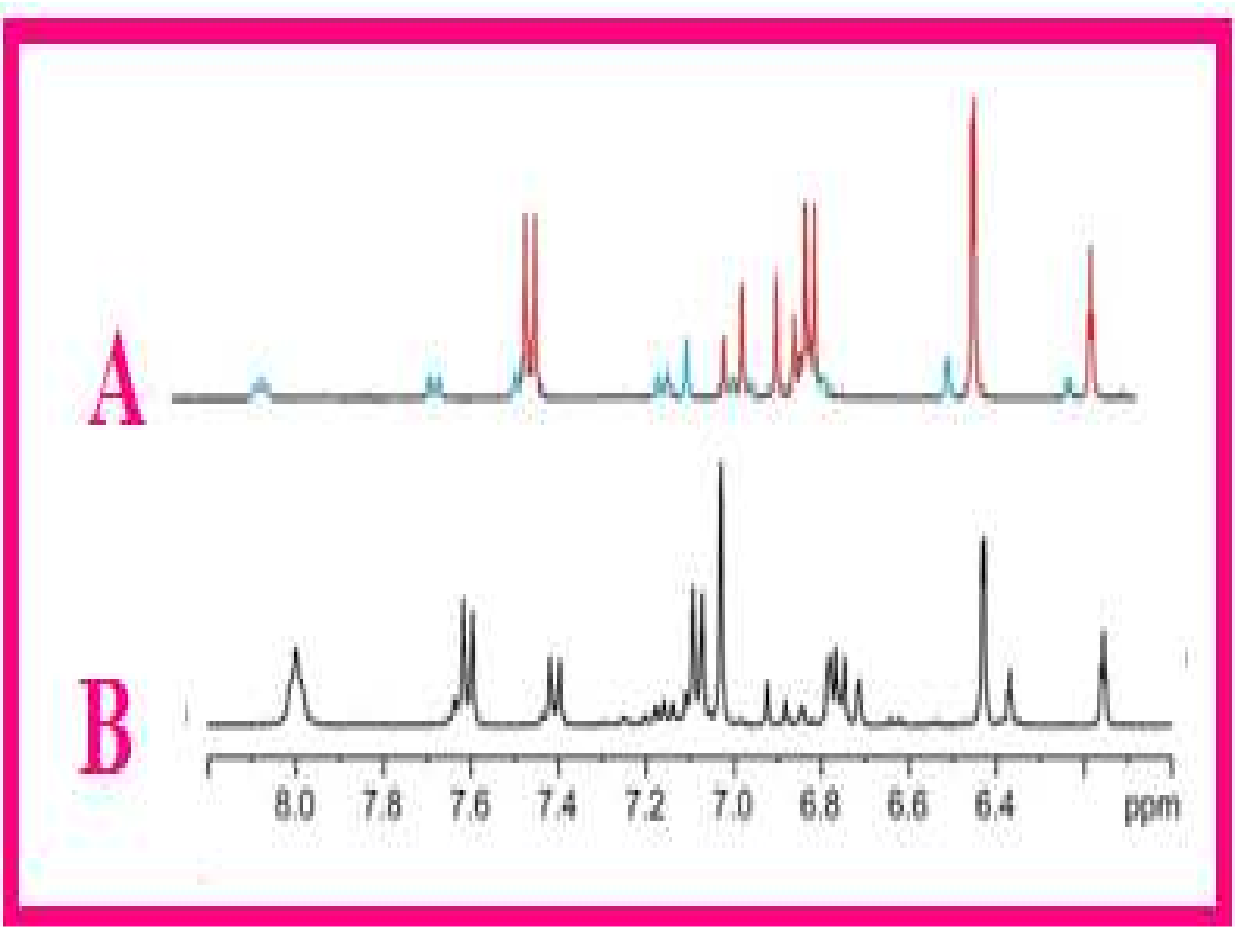



**Fig 3b: NMR spectra for trans (A) and cis (B)-Resveratrol**

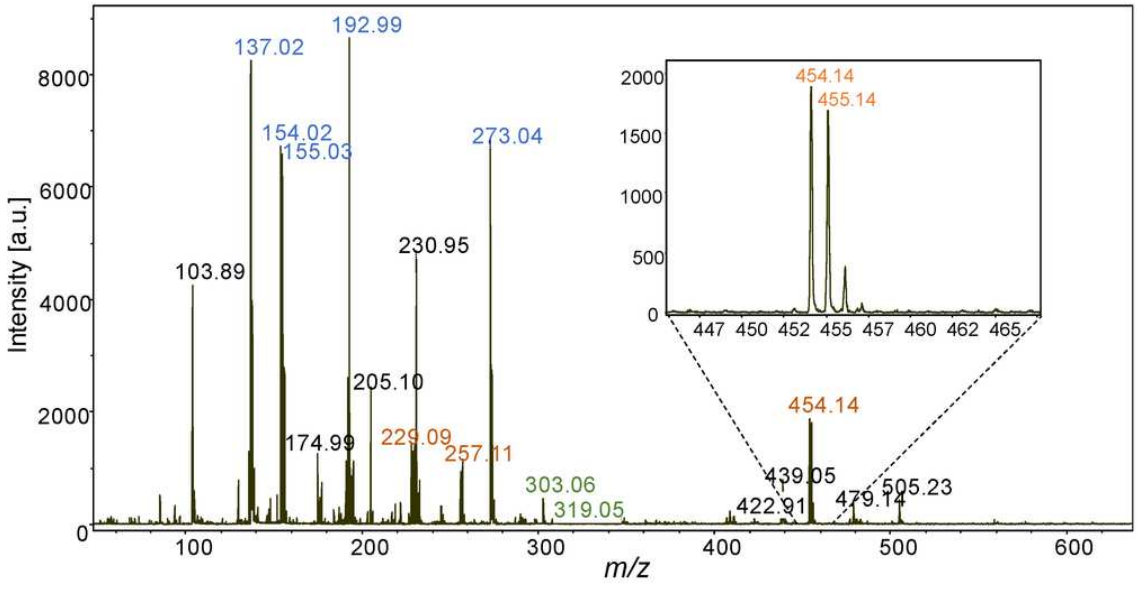



**Fig 4a: Mass spectra for Resveratrol**

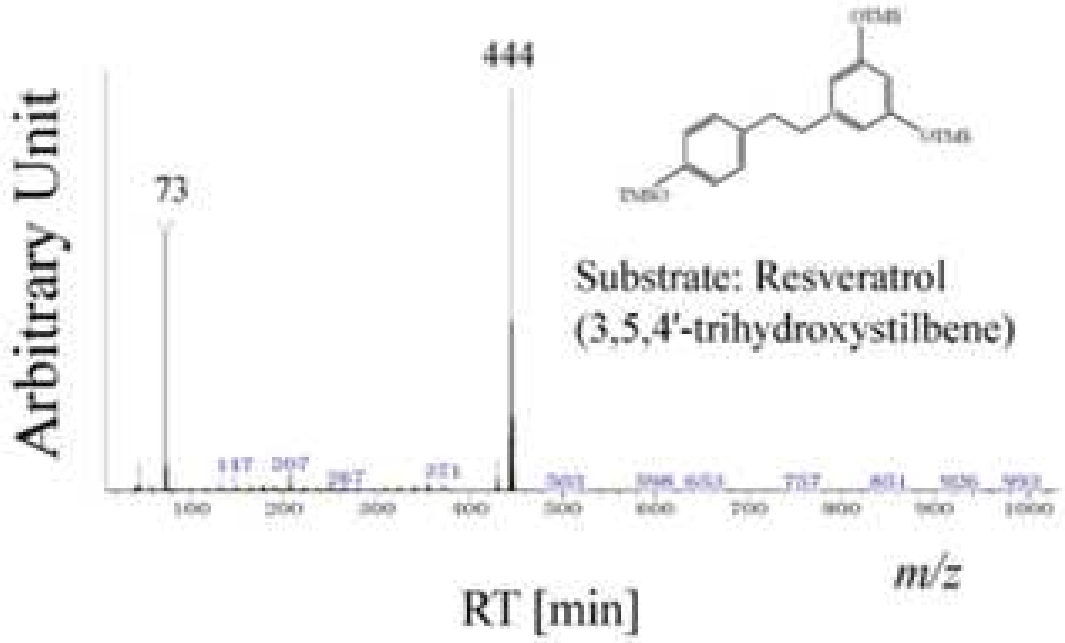



**Fig 4b: Mass spectra for Resveratrol (HPLC/MS/MS)**

**IR & UV Spectroscopy**

The UV spectrum was recorded on a Shimadzu UV-visible spectrophotometer using acetonitrile as the medium. The IR spectrum displayed characteristic absorptions at, 3422 (broad signal), 3027, 2929, 1511, and 1492 cm−1 which is indicative of O-H, aromatic C-H, methylene C-H, and aromatic –C=C stretching functionality.

**NMR spectroscopy**

The 1H chemical shift values were reported on the δ scale in ppm, relative to TMS (δ = 0.0 ppm) and in the $^{13}C$ NMR, the chemical shift values were reported relative to DMSO-d6 (δ = 39.50 ppm) as a reference. The DEPT-135 spectra revealed the presence of methyl and methine groups as positive peaks and methylene as negative peaks. 1H NMR data indicate the alkene hydrogens at the C7 and C8 positions observed as two doublets at δ 6.25–6.35 ppm with a coupling constant J1,3 =12.28 Hz which confirms the cis orientation of the two alkene hydrogens [14]. In trans-resveratrol, these two alkene hydrogens were observed at around δ 6.79–6.90 ppm with a coupling constant J1,3 = 16.2 Hz. The three hydroxyl groups were observed as broad signals at δ 9.19 (2H's) and 9.47 ppm, which disappear on D2O exchange studies. The aromatic hydrogen between the two hydroxyl substituents at the C4 position was found to be shielded to δ 6.05 ppm as a triplet with a meta coupling constant of J1,3 = 2.1 Hz, similar to that of trans-resveratrol. The hydrogens at the 2 and 6 positions were shielded to δ 6.11 and appear as doublets with a coupling constant J1,3 = 2.2 Hz, which is a characteristic coupling constant for the aromatic hydrogens in the meta position .The remaining aromatic hydrogens at 10, 11, 13, and 14 appear as two doublets δ 6.60 & 7.06 ppm with a coupling constant of J1,3 = 6.7 Hz, which matches the coupling constant for the ortho coupling in aromatic systems, and also the splitting pattern matches the p-substituted aromatic compounds.

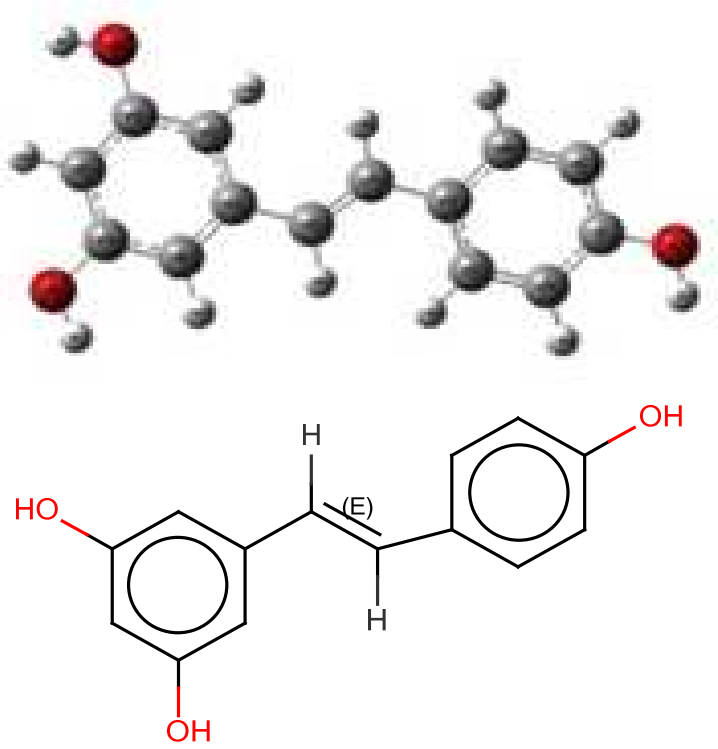
In the 13C NMR spectrum, three carbons deshielded more than the others and were observed as two sets at δ 156.7 and 158.4 ppm, which indicate that these carbons are attached to hydroxyl groups and assigned for the C3, C5, and C12 carbons. Due to the hydroxyl substituent effect at the ortho position, the carbons at C4, C6, C2, C11, and C13 appear more shielded at δ 101.6 (1C), 106.4 (2C), and 115.0 (2C) ppm. By using DEPT NMR data, all of the carbon signals were assigned. The NMR spectral data matches that reported for cis-resveratrol.

In dimethyl sulfoxide (DMSO), the 1 H-NMR data were as follows: 6.11 (m, 1H, H-4), 6.37 (d, 2H, J¼2.0 Hz, H-2, H-6), 6.76 (d, 2H, J¼8.5 Hz, H-3 0 , H-5 0 ), 6.90 (d, 1H, J¼16.3 Hz, H-a), 6.92 (d, 1H, J¼16.3 Hz, H b), 7.39 (d, 2H, J¼8.5 Hz, H-2 0 , H-6 0 ), 9.17 (s, 2H, 3-OH, 5-OH), 9.52 (s,1H, 4 0 -OH). From the above data, it was easy to confirm resveratrol.

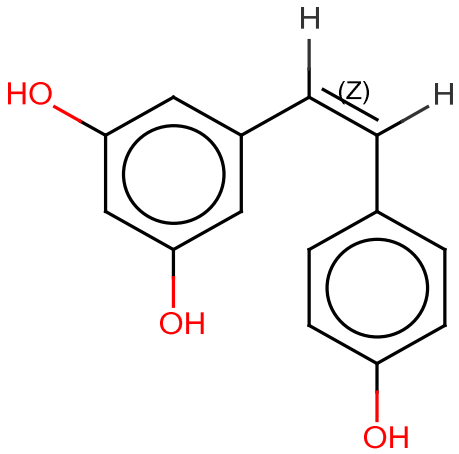
**Mass spectroscopy**

HPLC/MS/MS analyses were performed using a system consisting of a Finnigan autosampler, a Finnigan LC pump, a Finnigan TSQ Quantum Ultra equipped with an electrospray ion source and operated by XCalibur software. HPLC–ESI–MS/MS and was adopted to analyze trans-resveratrol. The Mass spectra of trans-resveratrol also recorded by JEOL GCmate. The data were shown here: parent ion $[M-H]^-$ m/z 227, main product ions m/z 185, 143, 117 and 119. The molecular mass of trans-resveratrol is 228.

**Structure of Resveratrol**




5-[(E)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol




5-[(Z)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol

**Evaluation of acute toxicity** [46]

In the present study the acute oral toxicity of the ethanolic extract **(ENL)** of black grapes (***Vitis vinifera***) was performed by acute toxic class method. In this method the toxicity of the extract was planned to test using step wise procedure, each step using three Wister rats. The rats were fasted prior to dosing (food but not water should be withheld) for three to four hrs. Following the period of fasting the animals were weighed and the extract was administered orally at a dose of 2000 mg/Kg b.w. Animals were observed individually after dosing at least once

during the first 30 min; periodically the surveillance was carried out for the first 24 hrs with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days.

**Experimental protocol for Hepatoprotetive activity** [47]
A total of 30 rats were taken and divided into 5 groups of 6 rats each
**(A) Group I:** Normal Control Group [**NCG -** (only the vehicle (1 mL/kg/day of 1% CMC; p.o.)].
**(B) Group II:** Negative Control Group **[Neg.CG** – (CCl4 1 mL/kg (1:1 of $CCl_4$ in olive oil) i.p].
**(C) Group III:** Positive Control/Standard Group **[SG -** $CCl_4$ 1 mL/kg (1:1 of $CCl_4$ in olive oil) i.p.+ Standard Silymarin 100 mg/kg orally (p.o.) for 7 days]

**Treatment Groups**
**(D) Group IV:** High Dose Group [**HDG** - $CCl_4$ 1 mL/kg (1:1 of $CCl_4$ in olive oil) i.p + ENL extract of BG **(Black grapes)** (500 mg/ kg b. w., p.o.)]
**(E) Group V:** Low Dose Group [**LDG** - $CCl_4$ 1 mL/kg (1:1 of $CCl_4$ in olive oil) i.p + ENL extract of BG (250 mg/ kg b. w., p.o.)]. Treatment was given daily for seven days orally.

**Collection of blood:** On the 8th day, blood was collected by retro orbital puncture, under mild ether anesthesia after 8 hr fasting. Blood samples were centrifuged at 3000 rpm for 20 mins. Serum was separated and stored at – $200^0$ C until biochemical estimations.

**Biochemical Analysis**
The Serum samples were analyzed for
(I ) Alanine Aminotransferase (ALT) (SGPT)
(II ) Aspartate Aminotransferase (AST) (SGOT)
(III) Alkaline Phosphatase (ALP)
(IV) Serum Bilirubin

**Histopathological Analysis**
The liver tissue was dissected out and fixed in 10% formalin solution. It was then dehydrated in ethanol (50%-100%), cleared in xylene and embedded in paraffin wax. Afterwards thick sections (5-6 mm) were made and then stained with hematoxylin and eosin dye for photo microscopic observation. The whole biochemical and histopathological analysis was carried out at V.H.S Hospital in Chennai.

**Evaluation of *in vitro* Cytotoxic activity by SRB Assay**
**Principle** [48, 49]
Sulphorodamine B (SRB) is a bright pink aminoxanthine dye with two sulfonic acid group. Under mild acidic conditions SRB dye binds to basic amino acid residues in trichloro acetic acid (TCA) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of visible at least two order of magnitude.

**Procedure** [50]
The monolayer cell culture was trypsinized and the cell count was adjusted to 0.5-1.0x105 cells/ml using medium containing 10% new born sheep serum. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately) 10,000 cells was added. After 24 hrs, when a partial monolayer was formed, the supernatant was flicked off, washed once and **100 µl, 50µl and 25µl and 12.5µl (in case of HT 29 Cell line) and 150µl, 100µl, 50 µl and 25 µl (in case of HS 578T Cell line)** of different concentration of extracts of fruits of Vitis vinifera were added to the cell in microtitre plate. The plates were incubated at 370c for 72 hrs in 5% CO2 incubator, microscopic examination was carried out and observations were recorded every 24 hrs. After 72 hrs, 25µl of 50% TCA was added to wells gently such that it forms a thin layer over the test extracts to form overall concentrations 10%. The plates were incubated at 40c for 1 hr. The plates were flicked and washed five times with tap water to remove traces of medium sample and serum and were then air dried. The air dried plates were stained with 100 µl SRB and kept for 30 mnts at room temperature. The unbound dye was removed by rapidly washing four times with 1% acetic acid. The plates were then air dried. 100 µl of 10 mM Tris base was then added to the wells to solubilise the dye. The plates were shaken vigorously for 5 mnts. The absorbance was measured using microplate reader at a 540 nm. The % growth inhibition was calculated by the following formula:

$$\% \text{ cell growth inhibition} = 100 - \{( At - Ab / Ac - Ab)\} \times 100$$

**At = Absorbance value of test compound.**
**Ab = Absorbance value of blank.**

**Ac = Absorbance value of control.**

## RESULTS AND DISCUSSION

**Table: 1 for the assessment of Biochemical parameters**

| Group | Treatment | AST(SGOT) IU/L | ALT(SGPT) IU/L | ALP(SALP) IU/L | Sr. bilirubin mg/dL |
|---|---|---|---|---|---|
| 1. | NCG | 52.53±1.065 | 48.63±1.162 | 51.97±2.313 | 0.7267±0.1443 |
| 2. | Neg. CG | 201.9±1.598*** | 209.5±2.858*** | 392.2±3.208*** | 7.300±1.012*** |
| 3. | LDG | 187.9±1.489*** | 160.5±12.31*** | 358.7±2.028*** | 4.633± 0.2603*** |
| 4. | HDG | 152.4±1.811*** | 122.5±13.84*** | 303.0±2.082*** | 3.397±0.3531* |
| 5. | SG | 91.60±1.795*** | 109.8±6.818*** | 155.7±6.240*** | 0.9000±0.0577ns |

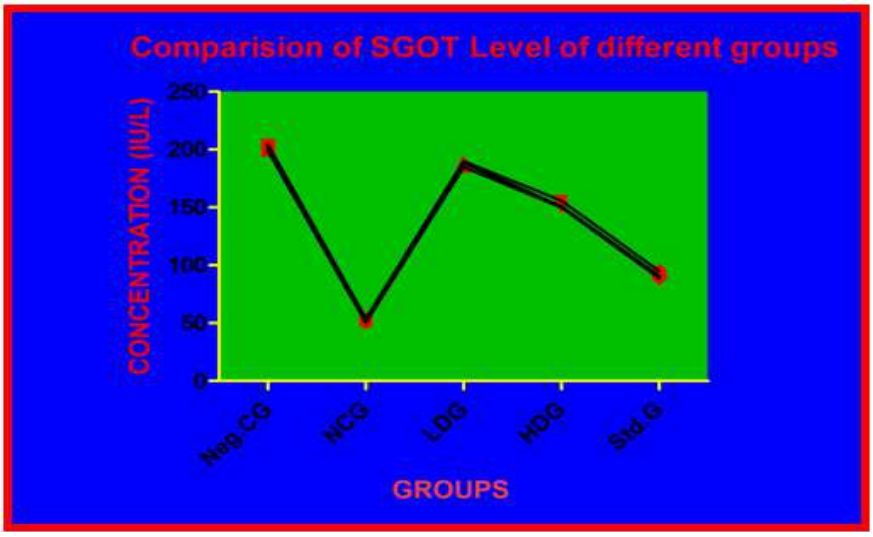



**Fig: 1**

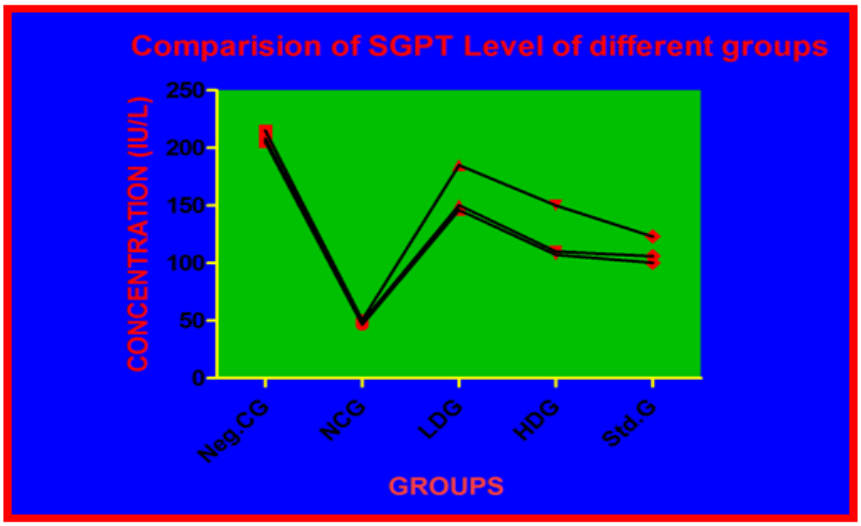
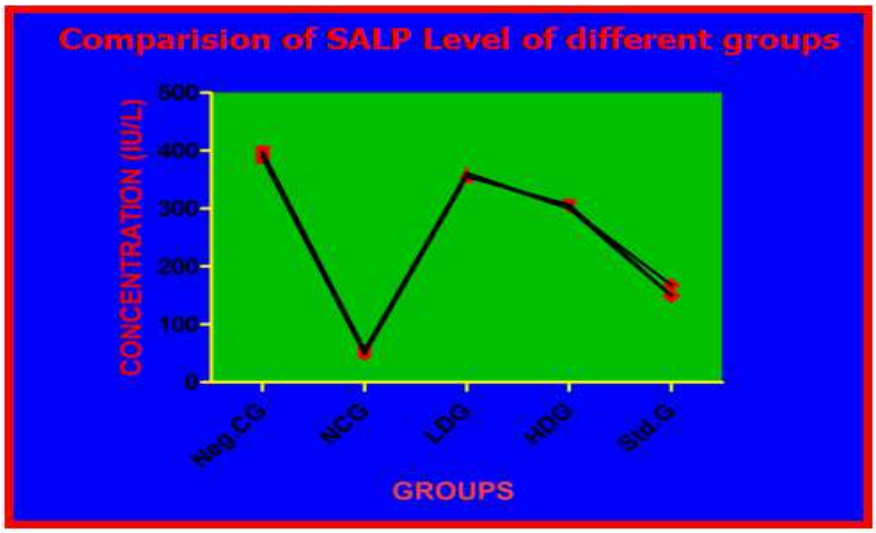



**Fig: 2**

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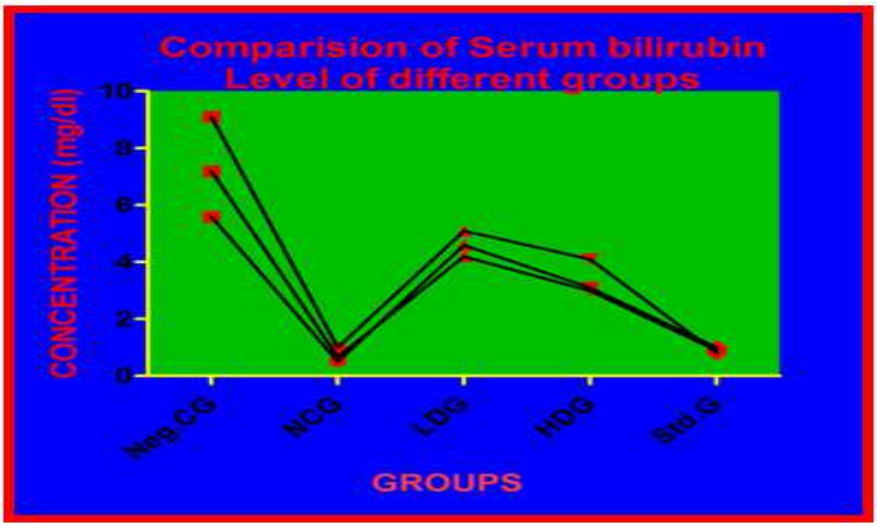


**Fig: 4**

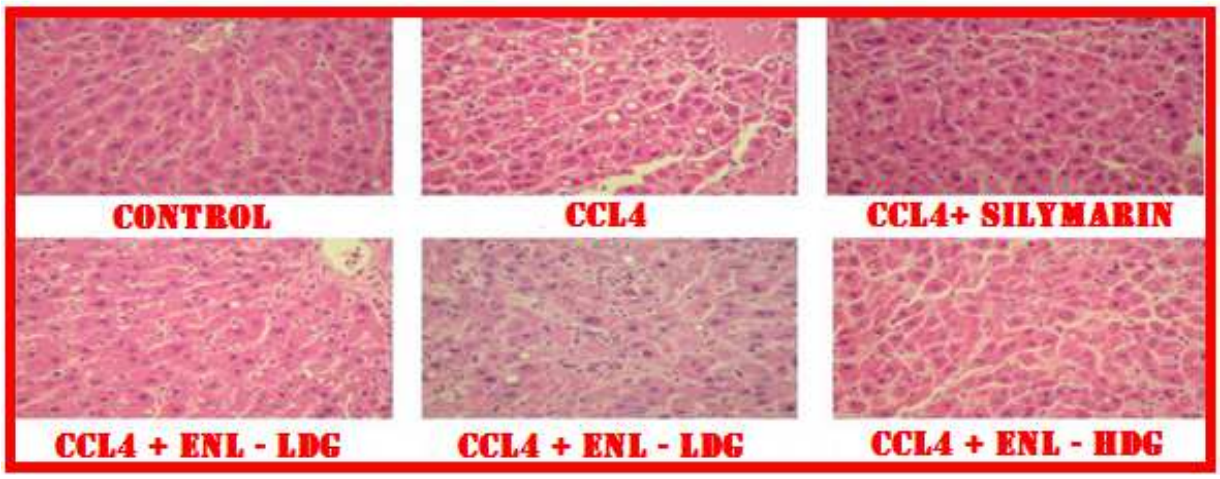


**Fig 5: Histopathological Examination of Hepatocytes treated with ethanolic extract Black grapes containing RVTL (BIOSPY)**

**Phytochemical screening**

Preliminary Phytochemical screening of ENL extract of black grapes (***Vitis vinifera***) had shown the presence of various bioactive compounds such as carbohydrates, aminoacids and peptides, phytosterols, carotenoids and polyphenols (higher concentration) etc.

**Acute Oral Toxicity Studies**

In this study the acute oral toxicity was evaluated by "Acute toxic class methods (OECD guideline-423)". The extract was administered orally at a dose of 2000 mg/Kg b. wt. During the surveillance period no significant toxicity occurred along with minute non-considerable behavioral changes. After a statistical analysis by trial and error, the significant doses were chosen at 250 mg/kg b. wt p. o (LD) and 500 mg/kg b. wt p. o. (HD) considerably.

**Hepatoprotective activity**

**Statistical analysis**

The data were expressed as mean ± SD. Statistical differences at *P < 0.05 between the groups were analyzed by one-way ANOVA followed by Dunnett's Multiple Comparison Test using Graph Pad Prism 5.04 Instate software package. The data's were compared with group 2 i.e. Negative Control group.

**Biochemical analysis**

The effects of ENL extract **of Black grapes (*Vitis vinefera*)** on liver marker enzymes and serum bilirubin content are displayed in **Table 1.** The data exhibited that Normal Control Group demonstrated a normal range of AST, ALT, and bilirubin levels while the $CCl_4$-treated group showed elevated levels of AST, ALT, and bilirubin, thus confirming that $CCl_4$ causes hepatocellular degeneration at higher doses. The elevation of cytoplasmic AST and ALT is considered an indicator for the release of enzymes from disrupted liver cells. Bilirubin concentration has been used to evaluate chemically induced hepatic injury. The Results displayed in **Table 1 and Fig: 1, 2, 3 and 4 were** indicated that the elevated levels of SGOT, SGPT, ALP and Serum bilirubin due to $CCl_4$ intoxication were reduced significantly (*P<0.05) in rats, after treatment with ENL extract **of Black grapes (*Vitis vinefera*)**. Treatment with **ENL extract of black grapes (*Vitis vinefera*)** at a both doses of **250 and 500 mg/kg b.w.** significantly decreased the SGOT, SGPT, ALP, Serum Bilirubin levels by **23.5%, 28.9%, 8.9%, and 17.2% (at low dose) and 26.9%, 35.9%, 14.5% and 22.3% (at high dose)** respectively. Silymarin used as standard drug showed a reduction of **56.09%, 69.89%, 57.46% and 19.04%**$^{ns}$ receiving $CCl_4$ alone. So depending upon the data of **Table 1** it was confirmed that the biochemical parameters of the group treated with ENL extract **of Black grapes (*Vitis vinefera*)** was significantly lower than the $CCl_4$-treated group. Moreover the treatment with the ENL extract **of Black grapes (*Vitis vinefera*)** significantly reduced the previously raised levels of AST, ALT, ALP and bilirubin in hepatotoxic rats.

**Histopathological Analysis**

The results of light microscopy examination of the transverse section of control, $CCl_4$-treated and treated with ENL extract **of Black grapes (*Vitis vinefera*)** rat livers were represented **in Fig 5.** It was revealed that the liver section of animals treated with $CCl_4$ showed a high degree of damage characterized by cell vacuolation, pyknotic and degenerated nuclei and wall of bile capillaries. The normal architecture of the liver was lost. The intralobular vein was badly damaged with wide spaces at some sinusoids. Liver sections of these rats indicated necrosis, ballooning and degeneration in hepatic plates and loss of cellular boundaries. There was also a heavy accumulation of neutrophils surrounding the portal vein. These neutrophils act as an indicator of the occurrence of cell damage as they are absent in normal healthy tissues. The hepatocytes are disrupted and sinusoids are damaged as well.

**Screening of cytotoxic activity**

The results for cell growth inhibition by the ENL extract containing **RVTL** of black grapes (***Vitis vinifera***) against **HT29 cell lines and HS 578T Cell line** at various concentrations was shown in table **1, 2**. As the concentration increases there is an increase in the cell growth inhibition and it was found that the highest **93.43%** growth inhibition at 12.5 µg (**$IC_{50}$ = 2.2 µg/ml**) against human colon cancer cell line HT-29 and **93.6%** growth inhibition at 25 µg (**$IC_{50}$ = 1.9 µg/ml**) against human breast cancer **HS 578T Cell line.** In the **USNCI screening program** a sample is generally considered to have ***in vitro* antineoplastic activity**, if the **$IC_{50}$ value** following incubation between 48 hrs and 72 hrs **is <4 µg/ml or 10 µM.** In the present study $IC_{50}$ values below **4 µg/ml** were displayed by ENL extract containing **RVTL** of black grapes (***Vitis vinifera***). The **$IC_{50}$** value of standard **drug 5-FU and doxorubicin** were found to be **1.91 µg/ml** with **96.54 %** growth inhibition at**1.5 µg/ml** and **1.81 µg/ml with 96.64%** growth inhibition at **2.5 µg/ml** respectively. Flavanoid and carotenoid compounds possessed the highest anticancer activity obtained from the natural sources.

**$IC_{50}$ Determination** [51]

$IC_{50}$ is the acronym for "half maximal inhibitory concentration". $IC_{50}$ value indicates the concentration needed to inhibit a biological or biochemical function by half (e.g. inhibition of enzymes, affinity to cell receptors). $IC_{50}$ is calculated by the following formula:

$IC_{50}$ = (50% - Low Inh%) / (High Inh% - Low Inh%) x (HighConc-Low Conc) + Low Conc.
Low Inh% / High Inh% : % inhibition directly below / above 50% inhibition

Low Conc / High Conc : Corresponding concentrations of test compound.

**Table 2: For percentage (%) of Cell Growth Inhibition by Ethanolic Extract (ENL) of Fruits of *Vitis vinifera* containing RVTL on HT-29 Cell lines by SRB Assay**

| Serial no. | Concentration of the Extracts | Absorbance of extracts | Inhibition of cell growth (%) |
|---|---|---|---|
| 1 | 12.5 µg/ml | 0.019 | 93.43 |
| 2 | 25 µg/ml | 0.022 | 92.3 |
| 3 | 50 µg/ml | 0.027 | 90.6 |
| 4 | 100 µg/ml | 0.033 | 88.59 |
| 5 | 1.5 µg/ml (5-FU) | 0.010 | 96.54 |
| 6 | Control | 0.289 | 0 |

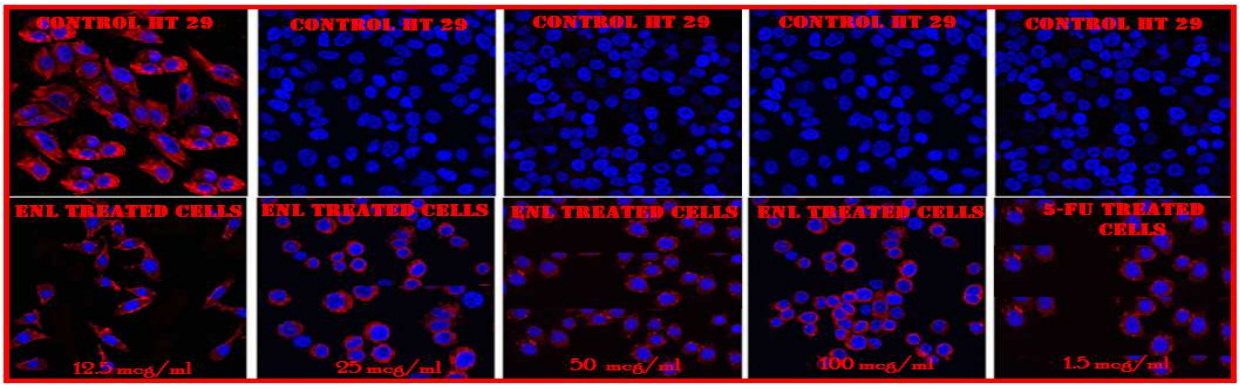


**Fig 6: Percentage (%) of cell Growth Inhibition by ENL extract of black grapes *(Vitis vinifera)* containing RVTL on Human Colon Cancer HT-29 Cell line**

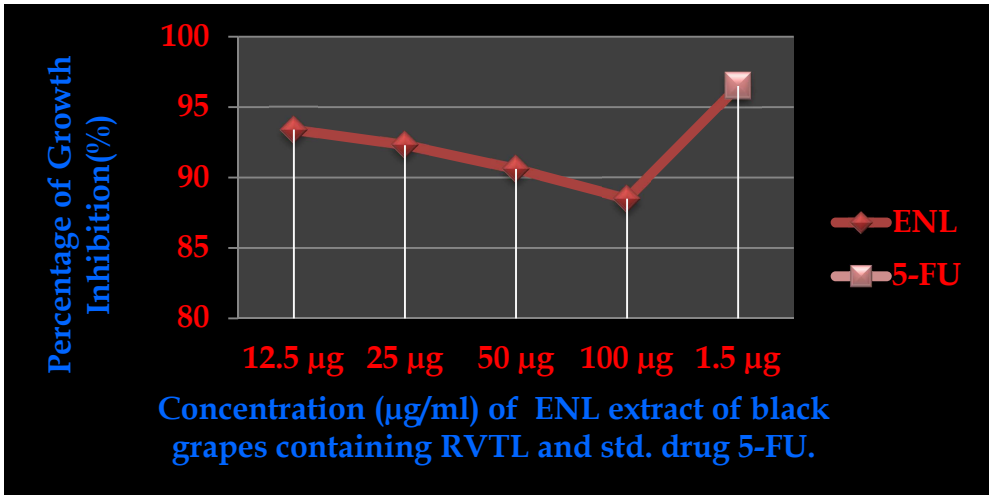


**Fig 7: Graphical representation of Percentage (%) of cell Growth Inhibition by ENL extract of Black grapes (*Vitis vinifera*) on HT-29 Cell line at different concentration**

**Table 3: For percentage (%) of cell Growth Inhibition by Ethanolic Extract (ENL) of Fruits of *Vitis vinifera* containing RVTL on HS-578T Cell line by SRB Assay**

| Serial no. | Concentration of the Extracts | Absorbance of extracts | Inhibition of cell growth (%) |
|---|---|---|---|
| 1 | 25 µg/ml | 0.019 | 93.6 |
| 2 | 50 µg/ml | 0.025 | 91.58 |
| 3 | 100 µg/ml | 0.034 | 88.56 |
| 4 | 150 µg/ml | 0.039 | 86.87 |
| 5 | 2.5 µg/ml (Doxorubicin) | 0.010 | 96.64 |
| 6 | Control | 0.297 | 0 |

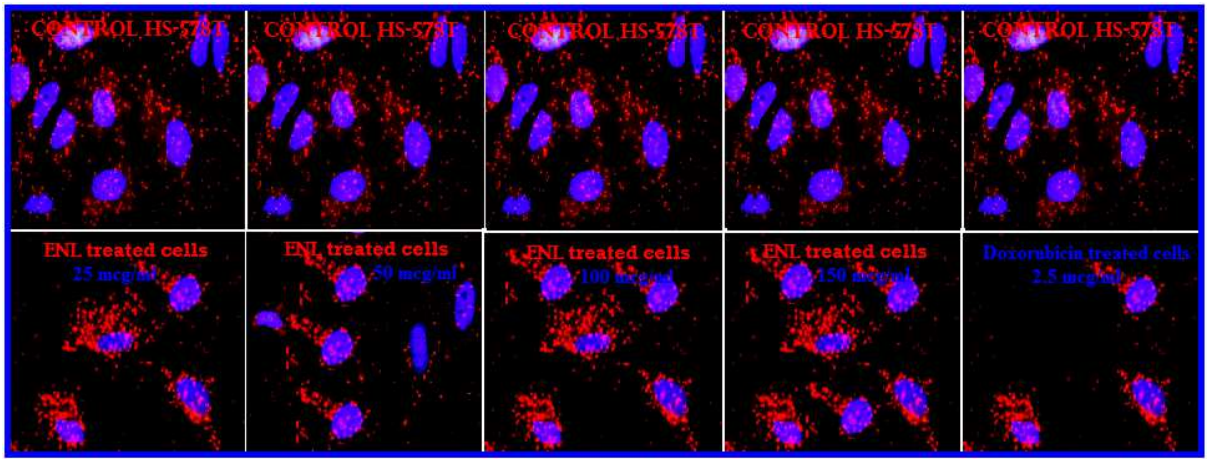


**Fig 8: Percentage (%) of cell Growth Inhibition by ENL extract of black grapes *(Vitis vinifera)* containing RVTL on Human breast Cancer HS-578T Cell line**

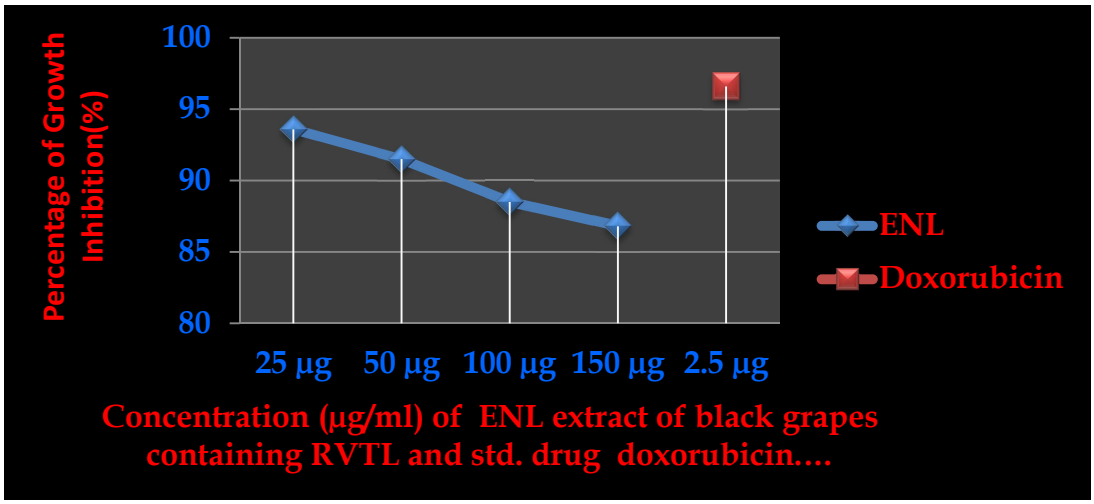


**Fig 9: Graphical representation of Percentage (%) of cell Growth Inhibition by ENL extract of Black grapes (*Vitis vinifera*) on HS-578T Cell line at different concentration**

## CONCLUSION

In conclusion, we report here that the **ENL extract containing RVTL** of black grapes ***(Vitis vinifera***) had ability to regenerate the hepatocytes *in vivo* and also possessed potential antiinflmmatory activity which was confirmed by liver biopsy. The hepatoprotective activity of the ENL extract of Black grapes (***Vitis vinefera)*** could be considered as excellent with regards to the standard drug silymarin.

The results obtained from the ***in-vitro*** studies performed by SRB assay using the **HT-29 cell lines and HS 578T** displayed that the **ENL extract containing RVTL of black grapes (*Vitis vinifera*)** possessed a very good antineoplastic activity. From the present studied it had been concluded that **ENL extract containing RVTL of black grapes (*Vitis vinifera*)** exhibiting the potential capability to kill the neoplastic cell when compared with standard drug **5-FU and doxorubicin**. The **ENL extract containing RVTL of black grapes (*Vitis vinifera*) displayed** with the highest **93.43%** growth inhibition at 12.5 µg (**$IC_{50}$ = 2.2 µg/ml**) against human colon cancer cell line HT-29 and **93.6%** growth inhibition at 25 µg (**$IC_{50}$ = 1.9 µg/ml**) against human breast cancer **HS 578T Cell line.**

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