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Diethylnitrosamine (DEN) Induced Hepatocellular Carcinoma

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ABSTRACT

The sixth most frequent malignant neoplasm worldwide is liver cancer, which continues to be a leading cause of mortality, particularly in South Western nations. Diethylnitrosamine, a dangerous environmental contaminant, is also known to cause cancer of the liver. According to reports, the drug vortioxetine (VT) is beneficial in preventing both carcinogenesis and different forms of chemically induced toxicity. In the current work, we assessed the effectiveness of VT as a chemopreventive agent against DEN-induced colon damage in a rat model. A biochemical assessment of antioxidant enzyme activities, lipid peroxidation, histopathological alterations, and expression of early molecular markers of inflammation and tumor promotion was used to assess the effectiveness of VT against liver damage. In the colons of Wistar rats, DEN therapy increased oxidative stress enzymes ($p<0.001$) and the early inflammatory and tumor-promotion response. Our findings suggest that VT significantly protects against chemically induced liver damage and likely exerts its protective effects through its antiproliferative properties.

INTRODUCTION:

The second most prevalent cause of cancer mortality and the fifth most common cancer diagnosed in males globally are liver cancer and liver disease, respectively (1). Of the main malignant tumors of the liver, hepatocellular carcinoma (HCC) makes up between 70 and 85 percent (2). The development of HCC is commonly linked to chronic liver inflammation brought on by ongoing hepatitis B and/or C virus infection (3). Recent epidemiological data show that HCC incidence is increasing in wealthy nations. This is explained by the rising incidence of hepatitis C virus infection and obesity-related diseases such as non-alcoholic fatty liver disease (4,5). Indeed, there is a sharp rise in the incidence and mortality of HCC in the USA (6). HCC still has a poor prognosis since there are no proven and efficient chemotherapy drugs for it, and its recurrence rate is significant.

In experimental animal models, diethylnitrosamine (DEN), sometimes called N-nitrodiethylamine, is frequently utilized as a carcinogen. Mice who are given DEN either orally or intraperitoneally develop a variety of cancers, including those of the liver, gastrointestinal system, skin, respiratory tract, and hematopoietic cells. By injecting DEN intraperitoneally (i.p.) into weaning mice at two weeks after birth, several researchers have used DEN to cause liver tumors in mice. These tumors appeared eight months later (7,8).DEN requires bioactivation by cytochrome P450 (CYP) enzymes in the liver because it lacks carcinogenicity of its own. As a result, DNA adducts develop via an alkylation process (9). A DNA repair gene called O6-methylguanine-DNA methyltransferase (MGMT) often referred to as O6-alkylguanine-DNA alkyltransferase can eliminate these alkylation adducts (10). Recently, Kang et al. (11) showed that for DEN-induced hepatocarcinogenesis, CYP2E1-deficient mice exhibit decreased tumor incidence and multiplicity compared to wild-type (WT) animals. Although various CYP enzymes have been hypothesized to catalyze DEN bioactivation in vivo, this data implies that CYP2E1 plays a crucial role in the activation of DEN (9).

An antidepressant drug called vortioxetine is used to treat major depressive disorder (MDD). It is categorized as a serotonin modulator and stimulator (SMS) because it acts on the serotonin neurotransmitter system in many ways, concurrently modulating one or more serotonin receptors and preventing serotonin reuptake. Vortioxetine specifically works through the following biological mechanisms: as a partial agonist of the 5-HT1B receptor, as an agonist of the 5-HT1A receptor, and as an antagonist of the 5-HT3, 5-HT1D, and 5-HT7

receptors. It also functions as a serotonin reuptake inhibitor (SRI) by inhibiting the serotonin transporter. There are several distinct subtypes of serotonin receptors; however, not all of these receptors appear to be involved in the antidepressant effects of SRIs, which is why SMSs were created. Others, such as 5-HT1A auto receptors and 5-HT7 receptors, appear to have an opposing function in the effectiveness of SRIs in treating depression. Some serotonin receptors appear to play a very neutral or minor role in the regulation of mood. (12)

This study aims to assess the effects of vortioxetine on liver cancer both in vivo and in vitro utilizing experimental animals and the hepatocellular carcinoma HepG2 cell line, which is most often utilized in drug metabolism and hepatotoxicity investigations. HepG2 cells execute a variety of differentiated liver activities, have high rates of proliferation, and are non-tumorigenic (13). Although vortioxetine has significant pharmacological significance, its chemotherapeutic potential as a complementary treatment for liver cancer illness is still unreported. Our results may provide scientific evidence for the therapeutic potential of this Medicine on liver cancer.

MATERIALS AND METHODS:

Vortioxetine and 5-Florourocil were obtained from Yarrow Chem Pvt. Ltd., Mumbai. All other chemicals used were of analytical grade obtained from Glaxo Laboratories, BDH division; Mumbai; India, and SD fine chemicals, Mumbai, India. In this research, male Wistar strain albino rats aged 6 to 10 weeks and weighing between 100 and 180g were employed. The animals were acquired through internal breeding at Lucknow's Aryakul College of Pharmacy and Research's Central Animal House Facility. The animals underwent a 12-hour day/night cycle during the duration of the trial, and the animal area was adequately ventilated. The animals were kept in roomy, capacious polypropylene cages and were allowed unlimited access to food and water. Before the trial began, the rats were acclimated for at least a week. The study has received approval from the institutional ethics committee, proposal/IAEC number 1896/PO/Re/S/16/CPCSEA/2021/1.

IN VITRO CELL LINE STUDIES

Cell Lines: Cell Repository at National Centre for Cell Science, Pune, provided the human hepatocellular carcinoma cell line (HepG2). The Aryakul College of Pharmacy and Research in Lucknow, Uttar Pradesh, India, maintains the cells.

Cell viability determination by trypan blue staining: One of the several stains suggested for use in dye exclusion techniques for viable cell counts is trypan blue. This technique is based on the idea that, in contrast to dead cells, living cells do not absorb dye. The Kugawa et al. technique was used to determine trypan blue exclusion. To count cells, trypan blue (0.4 percent) was dissolved in PBS. A tube containing 0.2 ml of trypsinized cell solution was filled with 0.3 ml of medium, which was then mixed with 0.5 ml of 0.4 percent trypan blue. Cells were counted on a hemocytometer after 5 minutes. The number of cells that weren't trypan blue-stained was counted as viable cells. (14)

Percentage of viability = Number of unstained cells/ Total number of cells x 1 00

***INVIVO* STUDIES**

Experimental design: The Male albino Wistar rats were uselessly alienated into five groups after successful induction of Liver cancer, and each group covers 5 rats. The groups of the animal are as follows:

- Group I: Sham control (received CMC)
- Group II: DMH control (40 mg/kg DEN)
- Group III: DMH control (40 mg/kg DEN) + SA (5-flourourocil) (50 mg/kg)
- Group IV: DMH control (40 mg/kg DEN) + VT (10 mg/kg) and
- Group V: DMH control (40 mg/kg DEN) + VT (20 mg/kg), respectively.

Estimation of Alpha-fetoprotein: Alpha-fetoprotein (AFP) was measured quantitatively by solid phase enzyme-linked immunosorbent assay (ELISA).

Hematological indices: (15)

Estimation of Red Blood Cell count: Red cell count was done according to the method of Chesbrough and McArthur (1972) in an improved Neubauer chamber. The RBC count is expressed as millionlmrn3 blood.

Estimation of White Blood Cell count: White blood cells were counted as the total number of cells per cubic millimeter of blood by the method of Chesbrough and McArthur (1972) using an improved Neubauer chamber. The WBC count is expressed as the total number of cells/mm3 blood.

Estimation of Hemoglobin: Hemoglobin content in blood was estimated by the method of Drabkin and Austin (1932). The value of hemoglobin is expressed as g/dl blood.

Estimation of blood Glucose: Blood glucose was estimated by the method of Sasaki and Matsui (1972). The values are expressed as mg/dl blood.

Estimation of Total Protein: Total protein was estimated by the method of Lowry et al., (1951). The level of protein is expressed as mg/g wet tissue for tissue and mg/dl for serum.

Estimation of renal function parameters in serum and urine (16, 17)

Estimation of Albumin: Albumin was estimated by the method of Fine (1935). The results are expressed as mg/dl in serum and µg/24 hrs in urine.

Estimation of urea: Urea was estimated by the method of Natelson et al., (1951). The results are expressed in mg/dl for blood and as mg/24 hrs for urine.

Estimation of uric acid: Uric acid was estimated by the method of Caraway (1963). The plasma uric acid level is expressed as mg/dl plasma. The urinary uric acid level is expressed as mg/24 hrs urine.

Estimation of creatinine: Creatinine was estimated by the method of Owen et al., (1954). Plasma creatinine level is expressed as mg/dl plasma. Urinary creatinine level is expressed as mg creatinine/24 h urine.

Estimation of total protein and albumin: Total protein and albumin in plasma were estimated by the method of Reinhold (1953). The difference between the total protein and albumin gives the globulin content in serum. The A/G ratio was calculated using the following formula.

$$\text{A/G ratio} = \frac{\text{Absorbance of albumin}}{\text{Absorbance of total protein} - \text{Absorbance of albumin}}$$

Total protein, albumin and globulin are expressed as mg/dl plasma.

Estimation of non-enzymic antioxidants in serum and tissue: (21)

Assay of Vitamin-C: Vitamin C was estimated by the method of Omaye et al., (1979). Vitamin C level is expressed as mg/g of wet tissue.

Assay of Vitamin-E: Vitamin E was estimated by the method of Quaife and Dju (1948). Vitamin E level is expressed as mg/g of wet tissue.

Estimation of phase I enzymes: (25)

Estimation of Cytochrome P450: Cytochrome P450 was estimated by the method of Omura and Sato (1964). The level of Cytochrome P450 was expressed as nmol/mg protein based on the molar-extinction coefficient.

Estimation of Cytochrome b5: The amount of Cytochrome b5 was measured by the method of Omura and Sato (1964). The level of Cytochrome b5 was calculated using the molar extinction coefficient of 185 mM/cm between 424-409 runs and was expressed as nmol/mg protein.

Estimation of NADPH cytochrome C reductase: NADPH cytochrome C reductase was determined according to the methods of Williams and Kamin (1962). The enzyme activity is expressed as moles of cytochrome c reduced/min /mg protein.

Estimation of NADH cyt bs reductase: Assay of NADH-cytochrome b5 reductase was carried out according to the method of Mihara and Sato (1972). The enzyme activity was calculated using the extinction coefficient of 1.02 mM/cm. The results are expressed as μmoles of NADH oxidized /min /mg protein.

Estimation of phase II enzymes (26)

UDP-glucuronyl transferase: The UDP-glucuronyl transferase was estimated by the method of Issalbacher, (1962) as modified by Hollman and Touster, (1962). The enzyme activity is expressed as nmoles/min/mg protein.

Assay of Glutathione-S-Transferase: The enzyme was assayed by the method of Habig eta/., (1973). Enzyme activity is expressed as 11moles of CDNB utilized/min/mg protein at 37 °C.

Histopathological studies: (27)

Morphological and histopathological studies were carried out in liver and kidney tissues of control and experimental rats. Liver samples were usually taken from the right portion of the median lobe as this site had more apparent histological lesions. Tissues were fixed in 10%

buffered formalin, routinely processed, and embedded in paraffin wax. Consecutive sections were cut at a thickness of 4µm and stained with hematoxylin and eosin (Culling et al., 1974).

Statistical Analysis:

The values are expressed as mean ± SD. The results were computed statistically (SPSS software package, version 7.5) using a one-way analysis of variance (ANOVA). Post hoc testing was performed for inter-group comparison using Student-Newman-Kuel multiple comparison test. Values of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

IN VITRO **STUDIES USING HUMAN HEPATOMA CELL LINE (HepG2):** To assess the inhibitory effects of Vortioxetine on growth rates, HepG2 cells were continuously cultured in the absence and presence of different concentrations (1, 10, 20, 50, 100 and 200 µg/ml) of the drugs at different time intervals. After 24, and 48h of treatment, the viability of the cells was determined by tryphan blue exclusion. The assay relies on the alteration in membrane integrity as determined by the uptake of the dye by dead cells. Trypan Blue is a vital dye. The reactivity of trypan blue is based on the fact that the chromophore is negatively charged and does not interact with the cell unless the membrane is damaged. Therefore, all the cells which exclude the dye are viable.

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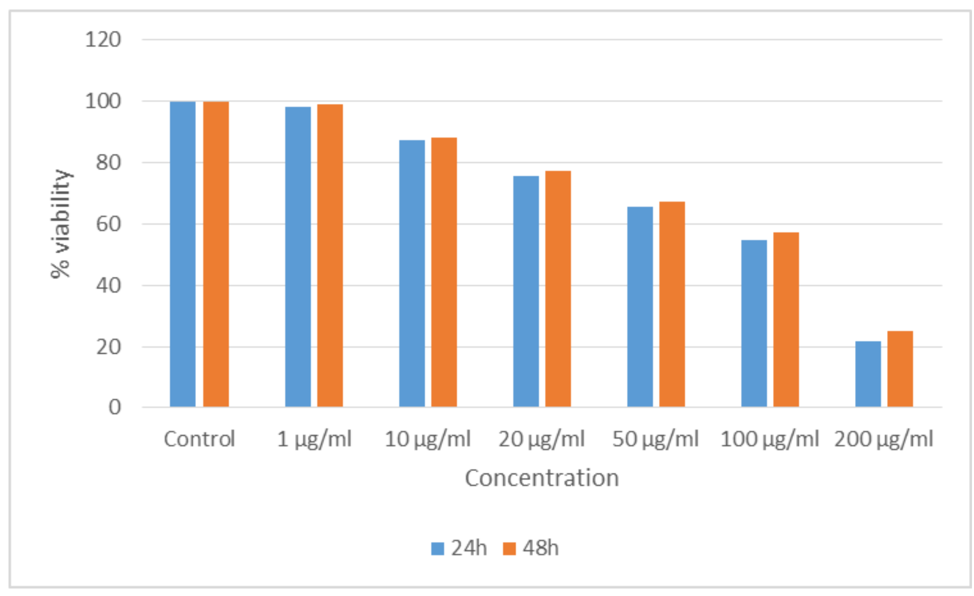


Figure 1: Cytotoxic effect of various doses of vortioxetine for 24h and 48h incubations

All doses of Vortioxetine inhibited cell proliferation in a dose-dependent manner; figure 1 shows good discrimination in cell inhibition. The cell viability of HepG2 cells decreased from 100% to 20% as the vortioxetine concentration increased from 1 to 200 µg/ml, after 24 and 48 hours of incubating at 37 °C. Based on the MTT assay results HepG2 cells treated with vortioxetine inhibitory concentration (IC50) at 20 µM were chosen as the optimum dose for further experiment.

PHARMACOLOGICAL AND THERAPEUTIC EFFECT OF VORTIOXETINE- *IN VIVO* STUDIES

Effect of Vortioxetine on the level of Alpha-fetoprotein (AFP): The effect of vortioxetine on the level of AFP is shown in Table 1. AFP level was found to be significantly ($p<0.05$) elevated in DEN-induced Group II animals when compared to Group I animals. Administration of vortioxetine significantly ($p<0.05$) decreased the levels of AFP in tumor-bearing animals than SA.

Effect of Vortioxetine on Body and Organ Weight Changes: The body weight changes of control and experimental animals are shown in Table 1. Decreased body weight changes were observed in DEN-induced animals. The body weight of the animals steadily increased significantly ($p < 0.05$) after treatment with SA (Group III) and VT (Group IV) animals. Group V drug control animals did not show any significant ($p<0.05$) variation in body weight when compared to the normal control. A significant difference ($p<0.05$) in body weight was observed when SA (Group III) and VT-treated groups (Group IV) were compared. As a VT drug, it possesses anticancer activity in DEN-induced HCC in experimental animals.

Effect of VT on Marker Enzymes in Liver: Table 2 depicts augmented activities of marker enzymes in serum viz., alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GT), lactate dehydrogenase (LDH) and 5 '-nucleotidase. In cancer-bearing animals, the levels of these enzymes in serum were significantly increased. This may be due to the discharge of those enzymes from damaged tissue membranes and release into the blood.

Table 1: Effect of Vortioxetine on AFP and Body Organ Weight

Parameters	Group I (Control)	Group II (DEN induced)	Group III (DMH + 5-FL)	Group IV (DEN+ 10mg/kg VT)	Group V (DEN+ 20mg/kg VT)
Body weight (g)	302.74 ± 25.86	163.96 ± 18.42^{a*}	289.55 ± 29.09$^{b*\ c\ NS}$	294.21 ± 32.71^{b*}	302.19 ± 37.97$^{d\ NS}$
ALF (IU/L)	4.98 ± 0.67	10.65 ± 1.56 a*	7.98 ± 0.86 b* c*	5.98±0.65^{b*}	5.34 ± 0.68 $^{d\ NS}$

Values are expressed as mean± SD for six animals. Comparisons are made between, 'a'- Group II Vs Group I, 'b'- Group III and IV, Vs Group II, 'c' Group IV Vs Group III, ' d' Group V Vs Group I. The symbol, *represents the statistical significance at $p < 0.05$, NS- Non-Significant.

Effect of VT on the Levels of Urea, Uric Acid, Creatinine, Haemoglobin, and Glucose: The content of hemoglobin, glucose, urea, uric acid, creatinine, and bilirubin in control and experimental groups of rats are presented in Table 2. Rats challenged with DEN showed a significant reduction ($p < 0.05$) in the blood hemoglobin, RBC, glucose, and uric acid levels, whereas WBC, serum creatinine, and serum urea levels were increased significantly ($p < 0.05$). These levels were brought back to near normal levels by treating the rats with SA and VT (Group III and IV). Drug control animals did not show any significant ($p <0.05$) changes.

Table 2: Effect of VT on the levels of urea, uric acid, creatinine, hemoglobin, and bilirubin

Parameters	Group I (Control)	Group II (DEN induced)	Group III (DEN + 5-FL)	Group IV (DEN+ 10mg/kg VT)	Group V (DEN+ 20mg/kg VT)
Urea	28.45 ± 2.22	49.22 ± 2.76^{a*}	39.56 ± 3.32$^{b*\ c}$	33.65 ± 2.76^{b*}	28.65 ± 2.87 $^{d\ NS}$
Uric Acid	1.54 ± 0.03	4.65 ± 0.94 ^{a*}	2.43 ± 0.06 $^{b*\ c*}$	1.96±0.35^{b*}	1.07 ± 0.08 $^{d\ NS}$
Creatinine	0.77 ± 0.07	1.78 ± 0.34^{a*}	0.89 ± 0.03$^{b*\ c}$	0.65 ± 0.04^{b*}	0.59 ± 0.13$^{d\ NS}$
Hemoglobin	6.65 ± 1.32	5.11 ± 1.01^{a*}	5.99 ± 1.22$^{b*\ c}$	5.05 ± 0.32^{b*}	4.98 ± 0.33$^{d\ NS}$

Values are expressed as mean± SD for six animals. Comparisons are made between, 'a'- Group II Vs Group I, ' be- Group III and IV, Vs Group II, 'c' Group V Vs Group III, 'd' Group V Vs Group I. The symbol, * represents the statistical significance at $p < 0.05$, NS- Non-Significant. Units: Hb - g/dl, Glucose, Urea, Uric acid, Creatinine and Bilirubin - mg/dl, RBC- x 10^6/mm\WBC - x $10^3/mm^3$

Effect of VT on albumin and globulin levels in the Liver: Table 3 shows the level of albumin and globulin in the serum of the control and experimental group of rats. A significant

reduction ($p<0.05$) in albumin level and a decrease in A/G ratio with a steep increase in globulin ($p<0.05$) levels were observed in DEN-induced animals (Group II) when compared to controls. On treatment with SA and VT (Group III and IV), significantly ($p<0.05$) the levels of these parameters were restored to a near normal state.

Figure 3: Effect of VT on the levels of Albumin, Globulin, and A/G ratio of liver of control and experimental animal

Parameters	Group I (Control)	Group II (DEN induced)	Group III (DEN + 5-FL)	Group IV (DEN+ 10mg/kg VT)	Group V (DEN+ 20mg/kg VT)
Urea	2.65 ± 0.23	3.76 ± 1.06^{a*}	2.45 ± 0.87$^{b* c}$	1.97 ± 0.76^{b*}	1.65 ± 0.65 $^{d NS}$
Uric Acid	1.2 ± 0.03	1.76 ± 0.94 ^{a*}	1.21 ± 0.05 $^{b* c*}$	0.99 ±0.05^{b*}	0.65 ± 0.02 $^{d NS}$

Values are expressed as mean± SO for six animals. Comparisons are made between, ·a' – Group II Vs Group I, 'b'- Group III and IV, Vs Group II, 'c' Group IV Vs Group III, 'd' Group V Vs Group I. The symbol, * represents the statistical significance at $p < 0.05$, NS- Non-Significant Units: Albumin; Globulin- g/dl

Effects of TD on Non-Enzymic Antioxidants: The activities of Vitamin C, Vitamin E, thiols, and GSH, in the liver, were shown in Table 4. The activities of non-enzymic antioxidants were decreased in DEN-induced animals. On VT and SA treatment, the levels of non-enzymic antioxidants were restored to near normal conditions. A significant difference ($p<0.05$) was observed when Group Ill and Group IV animals were compared. VT showed greater antioxidant activity than SA. No significant alteration of enzyme levels was observed in drug control animals when compared to Group I control animals.

Table 4: Effect of TD on enzymic antioxidants and thiols in the liver of control and experimental animals

Parameters	Group I (Control)	Group II (DEN induced)	Group III (DEN + 5-FL)	Group IV (DEN+ 10mg/kg VT)	Group V (DEN+ 20mg/kg VT)
Vitamin C	3.87 ± 0.49	1.97 ± 0.24 ^{a*}	3.56 ± 0.34 $^{b* c*}$	3.89 ±0.35^{b*}	4.22 ± 0.64 $^{d NS}$
Vitamin E	2.54 ± 0.32	1.54 ± 0.21 ^{a*}	1.98 ± 0.21 $^{b* c*}$	2.54 ±0.21^{b*}	2.45 ± 0.32 $^{d NS}$
TSH	9.45 ± 1.23	4.34 ± 0.65 ^{a*}	7.43 ± 0.54 $^{b* c*}$	8.23 ±0.65^{b*}	9.32 ± 0.45 $^{d NS}$
NPSH	5.99 ± 0.65	2.32 ± 0.43 ^{a*}	3.67 ± 0.32 $^{b* c*}$	5.32 ±0.23^{b*}	5.61 ± 0.55 $^{d NS}$

Units- GSH- mg/100g tissue, Vitamin C and E - mg/dl tissue, TSH and NPSH - µg/mg protein. Values are expressed as mean± SD for six animals. Comparisons are made between, 'a' - Group II Vs Group I, ' b' - Group III and IV, Vs Group II, ' c' Group IV Vs Group III, ' d' Group V Vs Group I. The symbol, *represents the statistical significance at $p < 0.05$, NS- Non-Significant.

Effect of VT on the Lysosomal Enzymes: Tables 5 depict the activities of lysosomal enzymes (~-glucuronidase, ~-D-galactosidase, N-acetyl -~-D-glucosaminidase (~ NAG), Acid phosphatase (ACP) and Cathepsin D) in the liver of control and experimental groups. A significant ($p < 0.05$) elevation is observed in the activities of lysosomal enzymes in DEN-induced rats (Group II) as compared to control rats (Group I). These enzyme activities decreased significantly ($p < 0.05$) when treated with SA and VT (Group III and IV). No significant ($p < 0.05$) changes were observed in rats treated with the drug 20mg/kg (Group II).

Table 5: Effect of VT on the activities of lysosomal enzymes in the liver of control and experimental animals

Parameters	Group I (Control)	Group II (DMH induced)	Group III (DEN + 5-FL)	Group IV (DEN+ 10mg/kg VT)	Group V (DEN+ 20mg/kg VT)
ACP	9.52 ± 0.99	17.87 ± 1.75^{a*}	13.34 ± 1.53$^{b*\ c}$	10.72 ± 1.12^{b*}	9.64 ± 0.89 $^{d\ NS}$
β-Glu	16.70 ± 1.09	32.46 ± 3.70^{a*}	23.55 ± 2.41$^{b*\ c}$	17.98 ± 2.94^{b*}	15.59 ± 1.67 $^{d\ NS}$
β-D-gal	15.78 ± 1.65	32.65 ± 4.23^{a*}	25.23 ± 3.05$^{b*\ c}$	18.45 ± 1.53^{b*}	14.89 ± 1.41 $^{d\ NS}$
β-NAG	24.65 ± 2.44	43.87 ± 4.56^{a*}	33.84 ± 4.11$^{b*\ c}$	27.87 ± 3.84^{b*}	23.12 ± 1.99 $^{d\ NS}$
Cathepsin D	28.97 ± 3.20	52.54 ± 5.12^{a*}	39.84 ± 3.98$^{b*\ c}$	32.63 ± 3.79^{b*}	28.28 ± 2.76 $^{d\ NS}$

Values are expressed as mean± SD for six animals. Comparisons are made between, ' a'- Group II Ys Group I, 'b'- Group III and IV, Ys Group II, ' c' Group IV Ys Group III, 'd' Group V Ys Group I. The symbol, *represents the statistical significance at $p < 0.05$, NS- Non-Significant.

Effect of VT on Biotransformation Enzymes: Tables 6 portray the alteration in the detoxification enzymes in the liver. In the present study, the activity of phase I enzymes namely, cytochrome P450, cytochrome b5, and NADPH cytochrome C reductase were found to be significantly ($p<0.05$) increased in liver tumor conditions. UDP-Glucuronyl transferase and GST were decreased significantly ($p<0.05$). These alterations were reverted to near

normal levels on drug treatment. There was no significant ($p<0.05$) difference between the control and drug-control animals.

Table 6: Effect of VT on the activities of detoxification enzymes in the liver of control and experimental animals

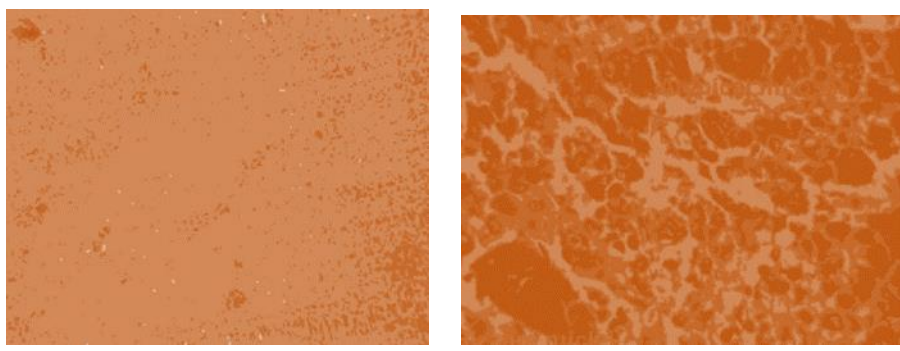
Parameters	Group I (Control)	Group II (DEN induced)	Group III (DEN + 5-FL)	Group IV (DEN+ 10mg/kg VT)	Group V (DEN+ 20mg/kg VT)
CYP450	3.34 ± 0.38	7.52 ± 0.62^{a*}	3.87 ± 0.53$^{b*\,c}$	4.11 ± 0.76^{b*}	3.32 ± 0.33 $^{d\,NS}$
Cyt b_5	2.43 ± 0.29	6.76 ± 0.78^{a*}	4.11 ± 0.67$^{b*\,c}$	3.65 ± 0.42^{b*}	2.19 ± 0.31 $^{d\,NS}$
NADPH cyt C red	8.32 ± 0.96	19.87 ± 1.20^{a*}	14.23 ± 2.05$^{b*\,c}$	9.31 ± 1.03^{b*}	8.49 ± 1.01 $^{d\,NS}$
UDP-Glucuronyl transferase	84.87 ± 8.78	53.11 ± 6.45^{a*}	67.43 ± 7.27$^{b*\,c}$	79.11 ± 8.96^{b*}	83.35 ± 9.02 $^{d\,NS}$
GST	15.65 ± 1.54	6.76 ± 0.98^{a*}	11.87 ± 1.98$^{b*\,c}$	14.84 ± 1.64^{b*}	15.21 ± 1.05 $^{d\,NS}$

Units: CYP_{450}- nmoles /mg protein, Cyt b5- nmoles/mg protein, NADPH cyt c reductase-nmoles of cytochrome C reduced/min/mg protein, UDP-glucuronyl transferase: units/ min/ mg protein; GST: as nmoles of CDNB-GSH conjugate formed/min/mg protein. Values are expressed as mean ± SD for six animals. Comparisons are made between, ' a'- Group II Vs Group I, ' b' - Group III and IV, Vs Group II, 'c' Group IV Vs Group III, 'd' Group V Vs Group I. The symbol,* represents the statistical significance at $p < 0.05$, NS- Non-Significant

Histopathological Study:

The histological alterations of the liver in the control and experimental groups are shown in Figure 2. Hepatocytes are organized in plates in the liver tissue of Group I control animals, and the central vein and portal triads are normal. The group II liver has extensive nodules of a tumor made up of round and polygonal cells with vesicular nuclei. Prominent nucleoli with distinct pleomorphism and hyperchromatism are organized primarily in a trabecular pattern but occasionally as nests and sheets. The cytoplasm of certain tumor cells is transparent. There are large tumor cells that are dispersed and have strange nuclei. There are several mitotic figures visible. Necrosis has spread across wide areas. Variable levels of fibrosis and portal inflammation are visible in the nearby normal liver. One of the patients had more severe fibrosis, piecemeal hepatocyte necrosis, and inflammation, which resulted in nodular growths that resembled cirrhosis. Hepatocytes were seen in plates in the liver of group IV

(DEN-induced VT treatment). Hepatocytes exhibited cytoplasmic vacuolization in certain cases. Tumor-related evidence was nonexistent. Group III normal liver tissue devoid of tumor traces. Hepatocytes in the group V (VT) animal's liver had normal morphology and architecture, much like in the group I control animals.



Control group DEN induced Group

Standard group (5-FL) Group VT 10mg/kg Group VT 20 mg/kg

Figure 2: Histopathology Study

CONCLUSION:

As a result, the biochemical, histological, and molecular indicators suggested by the current investigation convincingly depict the potential chemotherapeutic activity of vortioxetine against DEN-induced hepatocellular cancer. The findings showed that vortioxetine has strong anti-tumorigenic, hepatoprotective, and cytoprotective properties. This discovery offers a solid foundation for future mechanistic studies in chemotherapeutic development, clinical liver cancer investigations, research, and clinical practice. Therefore, how vortioxetine works as a chemotherapeutic drug open up new possibilities for treating liver carcinogenesis.

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