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Phytochemical Screening And Anticancer Efficacy Of Boerhavia Diffusa: A Study In Hepatocellular Carcinoma-Induced Rat Model

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ABSTRACT

Background: This study aims to investigate the phytochemical composition, antioxidant and anticancer activity of *Boerhavia diffusa* extract in rats with hepatocellular carcinoma.

Methods: To identify the essential bioactive components *Boerhavia diffusa* leaves were extracted using ethanol and subjected to GC-MS analysis. In male Sprague Dawley rats, hepatocellular cancer was induced with diethylnitrosamine and then with phenobarbitone. *Boerhavia diffusa* extract was administered to the induced rats with low dose (250 mg/kg) and high dose (500 mg/kg). Superoxide dismutase and catalase was measured to evaluate the antioxidant activity. Anticancer efficacy was measured by the changes in alpha-fetoprotein, white blood cells, haemoglobin, red blood cells and histopathological analysis of liver. GC-MS analysis confirmed the ethanolic extract of Boerhavia diffusa contained phytoconstituents like vitamin E, phytol and β-sitosterol.

Results: Hemoglobin, WBC and RBC were significantly decreased in DEN induced group and recovered in extract administered group. The treated groups showed significantly higher levels of antioxidant enzyme activity, particularly CAT (1.49±0.092) and SOD (0.15±0.001), suggesting increased oxidative defense. Higher alpha-fetoprotein levels (6.97±0.623 ng/ml) were found in the carcinoma induced group and considerably lower level (4.43±0.521 ng/ml) was observed following the extract administration. Microscopic examination of the liver revealed restoration of hepatocytes in treated rats with less inflammation and reduced necrosis.

Conclusion: This research suggests that the ethanolic extract of *Boerhavia diffusa* reveals notable antioxidant and anticancer properties, indicating its potential as a treatment for hepatocellular carcinoma. *Boerhavia diffusa* was used in traditional medicine for liver diseases, have scientific evidence by the presence of bioactive phytochemicals.

Keywords: *Alpha-fetoprotein, hepatocellular carcinoma, Boerhavia diffusa, β-sitosterol*

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the prevalent type of cancer globally and a major contributor to the cancer death rate. About 900,000 new liver cases were reported globally in 2020 and by 2040, world health organization (WHO) estimates that there can be an increase of over one million liver cases annually (Sung et al., 2021). Chemotherapy, radiotherapy and in chronic cases transplantation of liver was the conventional treatment procedure. Due to poor prognosis, significant side effects and delay in early diagnosis, the survival rate in liver cancer was minimal (Llovet et al., 2021). However, recently consumption of herbal drugs have increased by the presence of phytochemicals which shown to have cytotoxic and antiproliferative effect. *Boerhavia diffusa* also known as Punarnava in Sanskrit. It is an herbaceous plant belongs to the family Nyctaginaceae. It has proven action of antioxidant, anti-inflammatory, diuretic, anti fibrinolytic, hepatoprotective and widely used in Ayurvedic management (Mishra, 2014). The pharmacological actions of *Boerhavia diffusa* are due to the major bioactive compounds such as flavonoids, alkaloinds, sterols and saponins (Naidu, 2012). Recently, the therapeutic

effects of *Boerhavia diffusa* have attracted interest in hepatocellular carcinoma. The extract of *Boerhavia diffusa* has the capacity to control signaling pathways by its antioxidant capacity and regulates the cell proliferation and cell death causing cytotoxicity on malignant cells (Rawat *et al.*, 1997; Awad et al., 2008). However, the antiproliferative and antitumor mechanism of *Boerhavia diffusa* was not studied extensively. Hence, the lack of understanding of the anticancer activity in animal models was needed to explore the mechanism.

Increased oxidative stress and long term liver injury has been related to the condition of hepatocellular carcinoma. Growth of cancer was related to the oxidative stress, caused by the imbalance in the release of more amount of reactive oxygen species in the antioxidant defense system (Luangmonkong, 2018). Superoxide dismutase and catalase are essential in the maintaining normal physiology of cell and they reduce the destructive effect of reactive oxygen species (Ighodaro & Akinloye, 2018). Natural antioxidants presents in the plant source, prevents the oxidative stress by destroying free radicals and can be a therapeutic agent in carcinoma (Lobo et al., 2010). Hence, to identify the bioactive compounds in the leaves of *Boerhavia diffusa*, GC-MS was used. Previous research on *Boerhavia diffusa* also identified phytochemicals such as β-sitosterol, phytol and vitamin E (Mishra et al., 2014; Lomenick et al., 2015). Especially, β-sitosterol found in plants found to arrest the cell cycle in cancer cells and cause apoptosis (Awad et al., 2000; Chen et al., 2024).

The purpose of this study was to investigate the antioxidant and anticancer activities of ethanolic extract of *Boerhavia diffusa* in hepatocellular carcinoma model. Rats were induced liver cancer, by a known carcinogen, diethylnitrosamine (Park et al., 2009). Diethylnitrosamine produces oxidative stress and inflammation of liver tissue in rats causing liver cancer. This cancer model was suitable to explore the therapeutic effects of anticancer drugs (Mansour et al., 2019). The major findings of the study, in the extract treated group showed reduction in alpha-fetoprotein levels, increased levels of catalase and superoxide dismutase. With restoration of normal hepatocytes, we suggest that the ethanolic extract possess anticancer property. Our study attempts to provide scientific evidence for the use of *Boerhavia diffusa* as a treatment for liver cancer and to identify possible mechanism of action.

2. MATERIALS AND METHODS

Plant collection

Fresh *Boerhavia diffusa* leaves were obtained in the month of May and June, from Puducherry region, India. Identification and authentication of *Boerhavia diffusa* was done by a botanist. The leaves were cleaned thoroughly with tap water to remove surface debris and dust. Following cleaning, the leaves were left to dry in the shade for seven to ten days at room temperature, until the moisture content was sufficiently reduced. The dried leaves were finely ground using a mechanical blender and the resulting powder was kept in sealed box at 4^0C.

Soxhlet extraction was employed using ethanol as solvent. The thimble of the soxhlet apparatus loaded with 20 grams of powdered plant material. The extraction process was done for 24 hours with each solvent until the solvent in the apparatus become colorless. The extracts were concentrated by evaporating the solvents using a rotary evaporator at 40^0C in the reduced pressure. The concentrated extract was kept in a closed storage container at 4°C for subsequent phytochemical analysis and biological testing (Hossain et al., 2014).

Gas Chromatography - Mass Spectrometry

This technique was used to examine the extracts of *Boerhavia diffusa*. The analysis was performed using a GC system (436-GC Bruker) equipped with an RTX-5MS column (30m×0.25mm i.d×0.25µm film thickness) and 5% diphenyl/95%dimethyl polysiloxane. Helium served as carrier gas by a steady flow rate of 1ml/min. Split ratio be 10:1 and the injection volume was 1µL. Oven temperature was maintained at 110^0C, which was maintained for 3.5 minutes; temperature was increased 10^0C/minute to reach 200^0C. Then 5^0C was increased per minute to attain 280^0C. This temperature was maintained for 12 minutes. Run time was 40.5 minutes and the injector temperature was at 280^0C.

The MS detector was set at 70eV, electron impact ionization mode and the ion source temperature was set at 250^0C. A mass range scan of 50-500 m/z was used. The solvent delay was set to 3.5 minutes. National Institute of Standards and Technology (NIST) library, version 2011 was used to identify the compounds with the mass spectra. The relative quantities of the identified compounds were expressed as a percentage of total peak area (Casuga et al., 2016).

Animals

Male Sprague Dawley rats in good health (7-8 weeks old, weight 100-150g) were used in this study. After arrival, the animals were given 14 days to accimatize to the lab environment. They were kept in rice husk bedding in polypropylene cages with a 12-hour light/dark cycle in regulated environmental conditions (30-70% relative humidity, 24 ± 2^0C). The rats were fed with a regular pellet diet and had unrestricted access to water. The Institutional Animal Ethics Committee authorized the study protocol (Approval No. NCP/IAEC/2022-23/18) and all the experimental procedures were performed out in compliance with the guidelines of the CPCSEA.

Experiment groups

Rats were divided into five groups at random, each group were allotted six rats.

- Group I: Normal - Control (no treatment)
- Group II: DEN + PB (Diethylnitrosamine and phenobarbitone-induced liver cancer)
- Group III: DEN + PB + 5-Fluorouracil (Standard anticancer drug, 20 mg/kg intraperitoneally)
- Group IV: DEN + PB + *Boerhavia diffusa* extract (250mg/kg orally)
- Group V: DEN + PB + *Boerhavia diffusa* extract (500mg/kg orally)

To induce hepatocellular carcinoma (HCC), diethylnitrosamine (DEN) was used as a carcinogenic agent. DEN (200mg/kg) was diluted in normal saline and administered as single intraperitoneal injection to all experimental groups except Group I (control), starting on day 0 (Singh et al., 2018). After two weeks of DEN administration, hepatocarcinogenesis was promoted by providing the rats with 0.05% phenobarbitone (PB) in their drinking water for 16 consecutive weeks to enhance tumor formation and progression (Herren & Pereira, 1983). Following the 16-week carcinogenesis induction period, treatment protocols were initiated. Group I (control) and Group II (DEN + PB) were given normal saline throughout the study to serve as negative controls. Group III (DEN + PB + 5-Fluorouracil) received the standard anticancer drug 5-fluorouracil (20mg/kg), was given intraperitoneally twice daily for 28 days. Groups IV and V received oral administration of ethanolic extract of *Boerhavia diffusa*, in the dose of 250mg/kg and 500mg/kg, respectively, once daily for 28 days. Throughout the experimental period, the body weights of all animals were carefully monitored and recorded at regular intervals to assess the effects of treatments on overall health and potential toxicity.

Phytochemical Analysis

Compounds were quantified using GC-MS, their molecular weights and chemical structures were confirmed by the NIST library (Dantu & Kumar, 2011).

Hematological Parameters

Blood samples were collected under anaesthesia (thiopentone sodium, 40mg/kg), at the end of the experimental day. EDTA – coated tubes were used for collecting the samples for haematological analysis. Hemocytometer was used to assess the number of red blood cells, white blood cells and Sahli's hemoglobinometer method was used to estimate haemoglobin levels (Bedi & Mahajan, 2023).

Antioxidant Enzyme Assays

Liver tissue (0.5 g) was homogenized in 0.1M of phosphate buffer with a pH of 7.4, to prepare liver tissue homogenates. After centrifuging the homogenate for 10 minutes at 4^0C in 2000 rpm, the supernatant was utilized for antioxidant tests. Kakkar procedure was used to assess the activity of superoxide dismutase (SOD), by measuring the enzyme capacity to prevent epinephrine from autoxidation at 480nm (Kakkar et al., 1984). Sinha's method was used to evaluate the catalase (CAT) activity, which involves the breakdown of hydrogen peroxide at 570nm. Enzyme activity was measured as units/mg protein (Sinha, 1972)

Alpha-fetoprotein (AFP) Estimation

Enzyme Linked Immunosorbent Assay kit was used to assess the serum alpha-fetoprotein in accordance with the manufacturer's instructions. A 96-well plate precoated with AFP antibodies was taken and 100µL of serum was added to each well. The 3,3,5,5, Tetramethylbenzidine was added to the wells after an hour of incubation at room temperature. After ten minutes, the stop solution was added to stop the reaction. Microplate reader was used for measuring the absorbance at 450nm (Snell, 1973).

Histopathological Examination

Rats were killed by administering them an excessive amount of thiopentone sodium (50mg/kg). The livers were excised, weighed and preserved in neutral buffered formalin (10%). The liver tissues were embedded in paraffin wax and blocks were prepared. Rotary microtome was used to prepare the sections with thickness of 5-6µm and stained with eosin and hematoxylin for histopathological assessment. Liver sections were examined for pathological changes such as necrosis, inflammation, bile duct hyperplasia and disruption of hepatic architecture (Suvarna et al., 2019).

Statistical analysis

The mean ± standard error (SEM) was used to convey the data. One way analysis of variance (ANOVA) and Dunnett's t-test for post hoc comparisons were utilized for analysis. A p-value < 0.05 was considered to be statistically important. Version 20.0 of SPSS software was used for all statistical analysis.

3. RESULTS

Phytochemical Analysis of *Boerhavia diffusa*

The phytochemicals of ethanolic extract of *Boerhavia diffusa* was analyzed using GC-MS (Figure 1). Analysis exposed the presence of several biologically active compounds in ethanol extract. The major compounds identified were 3-O-methyl-d-glucose (83.16%), 17-Octadecynoic acid (2.40%), β-sitosterol (6.96%) and phytol (3.41%). Vitamin E (3.61%) was present in significant amount. 17-Ocatdecynoic acid, methyl ester (0.45%) was present in less amount (Table 1). These bioactive compounds, particularly β-sitosterol and vitamin E, have known antioxidant and anticancer properties.

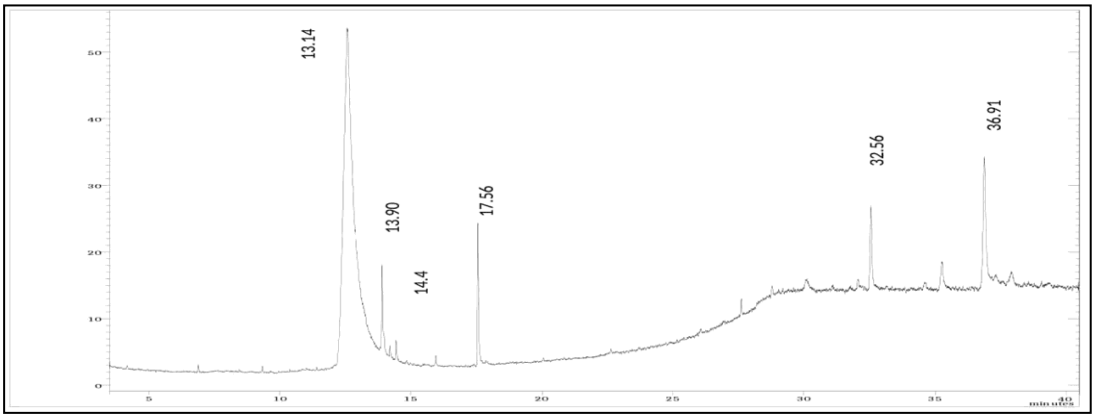
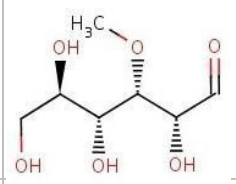
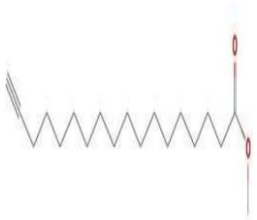
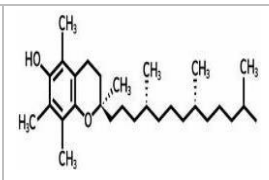
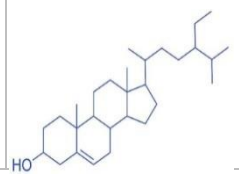


Figure 1: Chromatogram of ethanol extract of *Boerhavia diffusa*.

Table 1: Major compounds identified in ethanol extract of *Boerhavia diffusa* through GC-MS analysis.

No.	Retention time (min)	Name of the compound	Structure of compound	Molecular formula	Molecular weight	Peak area (%)
1	13.14	3-O-Methyl-d-glucose		$C_7H_{14}O_6$	194	83.16
2	13.90	17-Octadecynoic acid		$C_{18}H_{32}O_2$	280	2.40
3	14.44	17-Octadecynoic acid,methylester		$C_{19}H_{34}O_2$	294	0.45
4	17.56	Phytol		$C_{20}H_{40}O$	296	3.41

5	32.56	Vitamin E		$C_{29}H_{50}O_2$	430	3.61
6	36.91	β-Sitosterol		$C_{29}H_{50}O$	414	6.96

Hematological parameters

The influence of *Boerhavia diffusa* on haematological parameters in rats with DEN-induced hepatocellular carcinoma, were assessed by counting the number of red blood cells, white blood cells and hemoglobin levels (Table 2). In DEN-induced group (Group II), RBC levels significantly decreased compared to the control group (5.36 ± 0.277 vs. 5.48 ± 0.605 ×10⁶/µL). However, the low-dose (250 mg/kg) *Boerhavia diffusa* treatment (Group IV) restored RBC levels to control values (5.48 ± 0.368 ×10⁶/µL), while high-dose treatment (500 mg/kg, Group V) resulted in a slightly lower value (5.25 ± 0.192 ×10⁶/µL).

Table 2: Haematological Parameters in control, induced and treated group

GROUP	CONTROL	DEN+PB	DEN+PB+ 5-FLU	DEN+PB+ BD 250	DEN+PB+ BD 500
RBC ×10⁶/µL	5.48±0.605	5.36±0.277	5.87±0.106	5.48±0.368	5.25±0.192
WBC ×10³/µL	11.80±0.721	9.70±0.321	12.10±0.557*	10.00±1.04	10.50±0.85
Haemoglobin (g/dl)	13.70±0.41	12.9±0.12	14.4±0.233	13.40±0.41	12.00±0.353

WBC levels were also reduced in the DEN-induced group (9.70 ± 0.321 ×10³/µL) compared to controls (11.80 ± 0.721 ×10³/µL). The 5-fluorouracil-treated group (Group III) exhibited a significant increase in WBC (12.10 ± 0.557 ×10³/µL, $P<0.05$), and both low-dose and high-dose *Boerhavia diffusa* groups showed improvements in WBC levels (10.00 ± 1.04 ×10³/µL and 10.50 ± 0.85 ×10³/µL, respectively).

Similarly, significant reduction in the levels of haemoglobin in the DEN-induced group (12.9 ± 0.12 g/dl) compared to the control (13.7 ± 0.41 g/dl). Treatment with *Boerhavia diffusa* at 250 mg/kg (Group IV) improved hemoglobin levels (13.40 ± 0.41 g/dl), while the high-dose treatment group (500 mg/kg, Group V) showed a slight decrease (12.00 ± 0.353 g/dl) compared to the low-dose group.

Antioxidant Activity

Superoxide dismutase and catalase were assessed in liver tissue samples (Table 3). In the DEN-induced group, there was a significant reduction in SOD (0.08 ± 0.002 U/min/mg protein) and CAT (0.94±0.060 µmol of H_2O_2 consumed/min/mg protein) activities compared to controls (SOD: 0.19 ± 0.004 U/min/mg protein; CAT: 1.85±0.162 µmol of H_2O_2 consumed/min/mg protein). In the group treated with 5-fluorouracil, both SOD ($p<0.001$, 0.17±0.001 U/min/mg protein) and CAT ($p<0.001$, 1.63±0.122 µmol/min/mg protein) activities significantly improved.

Table 3: Antioxidant assay in control, induced and treated group

GROUP	CONTROL	DEN+PB	DEN+PB+5-FLU	DEN+PB+BD 250	DEN+PB+BD 500
SOD (Unit/min/mg/protein)	0.19±0.004	0.08±0.002	0.17±0.001***	0.13±0.003**	0.15±0.001***

CATALSE (µmol of H_2O_2 consumed/min/mg/protein)	1.85±0.162	0.94±0.060	1.63±0.122***	1.27±0.080**	1.49±0.092***

Similarly, in the low-dose *Boerhavia diffusa*-treated group, SOD activity increased significantly to 0.13±0.003 U/min/mg protein, p<0.01 and CAT activity was also enhanced (1.27±0.080 µmol/min/mg protein, p<0.01). The high-dose group (500 mg/kg) showed further improvements, with SOD activity reaching 0.15±0.001 U/min/mg protein, p<0.001 and CAT activity at 1.49±0.092 µmol/min/mg protein (p<0.001). These findings indicate a dose-dependent antioxidant effect of *Boerhavia diffusa*.

Alpha-fetoprotein (AFP) Levels

Alpha-fetoprotein (Table 4), a biomarker for hepatocellular carcinoma, was significantly elevated in the DEN-induced group (6.97 ± 0.623 ng/ml) compared to the control group (4.93 ± 0.504 ng/ml). In the group treated with 5-fluorouracil, AFP levels were significantly reduced (P< 0.05) to 4.90 ± 0.361 ng/ml, approaching normal levels. In the *Boerhavia diffusa* treatment groups, in low dose group, AFP levels declined modestly (5.60 ± 0.351 ng/ml) and a significant decrease (4.43±0.521 ng/ml, p<0.05) in high dose group was observed, in a dose-dependent method.

Table 4: Estimation of Alpha-fetoprotein in control, induced and treated group

GROUP	CONTROL	DEN+PB	DEN+PB+5-FLU	DEN+PB+BD 250	DEN+PB+BD 500
Alpha-fetoprotein	4.93±0.504	6.97±0.623	4.90±0.361*	5.60±0.351	4.43±0.521*

Gross appearance of liver tumors

After opening the abdomen of rats, liver was observed macroscopically for the appearance of tumors. In control group (Fig 2A), liver tissue shows normal texture and morphological feature without disruption of lobes. In DEN induced group (Fig 2B), liver found to have several nodules of tumor. 5-Flurouracil treated group (Fig 2C) showed reduction in number of tumors. Low dose *Boerhavia diffusa* extract treated group (Fig 2D) showed decrease in tumor size whereas the group treated with high dose of *Boerhavia diffusa* extract (Fig 2E) showed marked decrease in number of tumors.

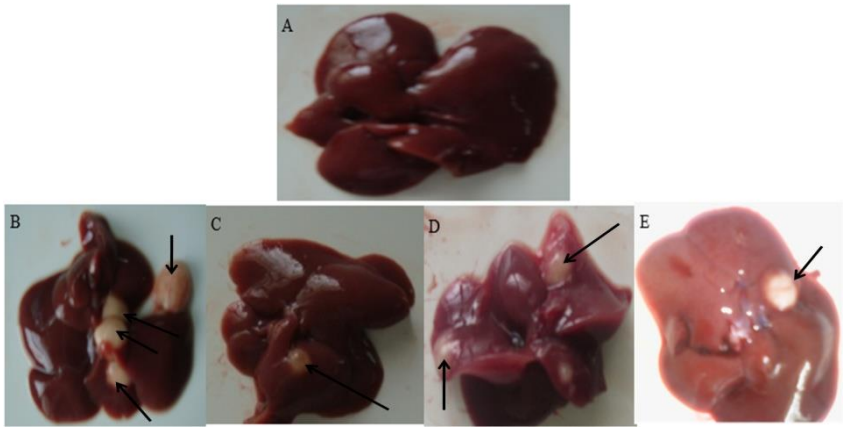


Figure 2: Gross morphological features of liver A. Normal group B. DEN induced group C. 5-FLU treated group D. Low dose BD treated group D. High dose BD treated group.

Histopathological analysis

Liver histopathological examination exposed significant pathological changes in the DEN-induced group. Liver sections from the DEN-induced group exhibited necrosis of hepatocytes with disrupted nuclear margins due to the accumulation of glycogen in cytoplasm, periportal inflammation, bile duct hyperplasia and dilated sinusoids due to loss of reticular fibers

(Figure 4 A). In contrast, the control group displayed normal hepatic architecture without signs of necrosis or inflammation (Figure 3). The 5-fluorouracil-treated group showed improved liver architecture with reduced necrosis and mild periportal inflammation (Figure 4 B).

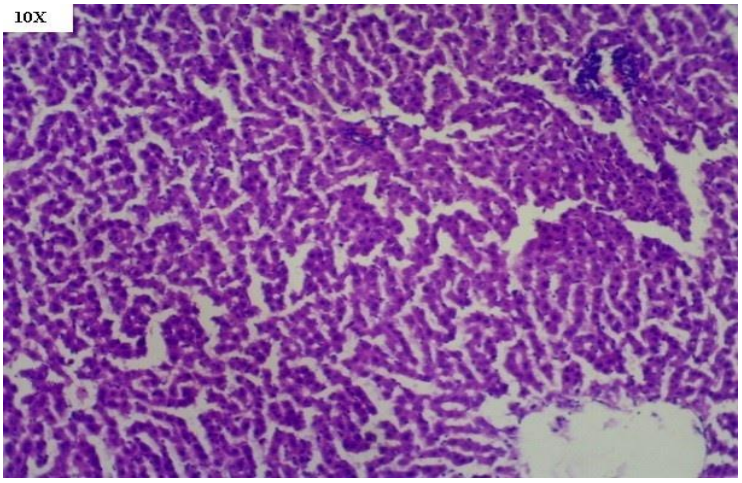


Figure 3: Histology of liver in control group

In the low-dose *Boerhavia diffusa* group (250 mg/kg), liver sections showed normal lobular architecture with mild periportal inflammation, binucleation of hepatocytes and minimal cytoplasmic vacuolation (Figure 4 C). The high-dose treatment group (500 mg/kg) exhibited near-normal liver architecture with restored hepatocyte structure, appearance of kupffer cells and minimal vacuolation indicating effective hepatoprotection (Figure 4 D).

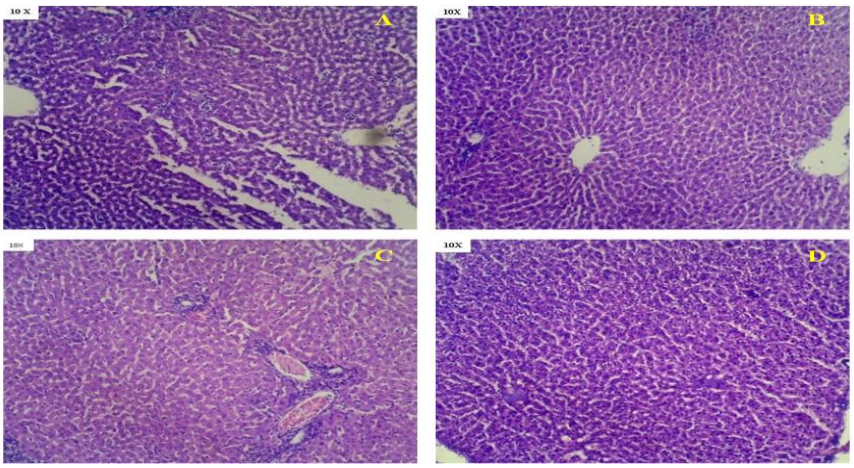


Figure 4: A. Histology of DEN induced group, B. Histology of 5-FLU treated group, C. Low dose BD treated group, D. High dose BD treated group.

4. DISCUSSION

The present study aims to examine the antioxidant and anticancer properties of an ethanolic extract of Boerhavia diffusa in hepatocellular carcinoma model induced by diethylnitrosamine. Based on the findings, *Boerhavia diffusa* had considerable hepatoprotective and antioxidant properties as evidenced by increased haematological parameters, reduction in alpha-fetoprotein levels and restoration of normal hepatocytes in liver tissue. The results reported here confirm the traditional use of Boerhavia diffusa as a herbal remedy for conditions related to liver and suggests its ability to treat cancer.

Phytochemical Composition and Bioactivity

GC-MS analysis identified the presence of bioactive compounds, such as Vitamin E, phytol and β-sitosterol which are known for their anticancer and antioxidant properties. The significance of the plant sterol β-sitosterol in treating cancer has been well studied. Previous studies in hepatocarcinogenesis mentioned the action of β-sitosterol in modifying the signaling pathways particularly Wnt/β-catenin pathway. β-sitosterol mainly arrest the multiplication of cancer cells and causes cell death. Another researcher found, β-sitosterol acts on FOXM1-regulated Wnt/β-catenin pathway and significantly affects the

spread and growth of hepatocellular carcinoma cells, suggesting its importance in the treatment of liver cancer (Chen et al., 2024). Phytol, a bioactive compound found in plants triggers oxidative stress and mitochondrial dysfunction in cancer cells. It also inhibits the growth of cancer cells and causes apoptosis, hence, phytol also have antioxidant and anti cancer properties (Dhaunsi et al., 2017). Our study aims to identify these bioactive substances in *Boerhavia diffusa* extracts further supports their role in the observed antioxidant and anticancer effects.

Hematological Parameters

Cancer patients found to have chronic inflammation, malabsortion and malnutrition and reduction in hematopoiesis leading to anemia with slow prognosis (Ludwig et al., 2013). In the present study, blood analysis in carcinoma induced group found to have considerable decline in haemoglobin, white blood cells and red blood cells, causing impaired hematopoiesis leading to anemia. However, treatment with *Boerhavia diffusa* at both low (250mg/kg) and high (500mg/kg) doses resulted in the restoration of RBC, WBC and hemoglobin levels to near-normal values. These findings are consistent with previous studies on the hematological benefits of *Boerhavia diffusa*. Another study demonstrated that *Boerhavia diffusa* has hematopoietic and immunomodulatory effects, which help restore normal hematological parameters in diseased models (Rawat et al., 1997). The restoration of these parameters in the current study suggests that *Boerhavia diffusa* extract not only exhibits anticancer properties but also alleviates the hematological side effects associated with HCC.

Antioxidant activity

The development and progression of liver cancer is significantly influenced by the oxidative stress by promoting DNA damage, inflammation, and uncontrolled cell proliferation. By neutralizing reactive oxygen species (ROS), the antioxidant enzymes superoxide dismutase and catalase provide the first line of defense against oxidative stress. In cancer induced group, both SOD and CAT activities were significantly reduced, reflecting oxidative stress and liver damage. However, treatment with *Boerhavia diffusa* significantly enhanced SOD and CAT activities, indicating its potential to restore antioxidant defense mechanisms.

Similar studies have corroborated the role of *Boerhavia diffusa* in mitigating oxidative stress. Mishra et al. demonstrated that *Boerhavia diffusa* ethanolic extract significantly increased SOD and CAT performance in various *in-vivo* and *in-vitro* models, suggesting that its antioxidant potential plays a key role in hepatoprotective effect (Mishra et al., 2014). Another study highlighted the importance of these antioxidant enzymes in cancer prevention, where a decrease in SOD and CAT levels corresponds to increased oxidative damage and carcinogenesis (Ighodaro & Akinloye, 2018). The current study findings align with these reports, emphasizing that the phytochemicals in *Boerhavia diffusa* are capable of scavenging free radicals and restoring antioxidant balance in the liver.

Alpha-fetoprotein (AFP) Levels

An elevated AFP level was found to be the major sign in hepatocellular carcinoma. Investigation of alpha-fetoprotein is important in diagnosis and prognosis of the disease. Hence, it is widely recognized as biomarker for HCC. In the present study, development of hepatocellular carcinoma in the DEN-induced group was confirmed by elevated levels of alpha-fetoprotein. Reduction in alpha-fetoprotein level was observed in both the low and high dose extract administered group. However, considerable decrease in high dose treated group was found when compared to the 5-fluorouracil administered rats. Significant decline of alpha-fetoprotein suggest that the extract possess strong anticancer effect. Similar study with *Boerhavia diffusa*, reported a decrease in alpha-fetoprotein and prevents the growth of malignant cells in hepatocellular carcinoma by the presence of β-sitosterol (Awad et al., 2000). Furthermore, another study demonstrated that β-sitosterol effectively suppressed tumor growth and metastasis, reducing AFP levels in HCC-bearing mice (Chen et al., 2024). The present study reinforces these findings, providing additional evidence that *Boerhavia diffusa* exerts its anticancer effects by modulating AFP levels and inhibiting tumor progression.

Histopathological Findings

The histopathological analysis in this study revealed significant liver damage in the DEN-induced group, characterized by necrosis, periportal inflammation, bile duct hyperplasia, and disruption of hepatic architecture. These findings are indicative of severe hepatocellular carcinoma development. However, treatment with *Boerhavia diffusa* extracts resulted in marked improvements in liver histology, with reduced necrosis, inflammation, and near-normal lobular architecture, particularly in the group receiving large dose (500 mg/kg). These histological changes imply that *Boerhavia diffusa* has hepatoprotective effects, which might be attributed to its capacity to scavenge reactive oxygen species and restore normal liver function.

In this study, in the extract administered group we found significant decrease in tumor size and reduction in number of tumors. Similar study in the DEN-induced hepatocellular carcinoma, the extract of *Boerhavia diffusa* effectively reduced the tumor volume and histology of liver hepatocytes was found to be normal (Kayande & Kushwah, 2014). Another study on *Boerhavia diffusa* reported, the damaged liver tissue was restored by reducing the inflammation and necrosis in experimental models (Das et al., 2023). The results obtained in this work support the findings with similar studies and suggest that *Boerhavia diffusa* possess strong antioxidant, anticancer and hepatoprotective properties.

5. CONCLUSION

In the present study, significant improvements were observed in red blood cells, white blood cells, SOD and catalase. Reduction in alpha-fetoprotein levels and restoration of normal hepatic architecture was also observed in high dose treated group. In addition, GC-MS analysis identified the potential bioactive compounds like Vitamin E and β-sitosterol. The findings suggest the extract possess antioxidant, anticancer and hematoprotective activity in hepatocellular carcinoma model induced by diethylnitrosamine. The pharmacological effect confirms the traditional use of *Boerhavia diffusa* as herbal drug in the treatment of hepatocellular carcinoma.

6. LIMITATIONS AND FUTURE DIRECTIONS

The current research on hepatocellular carcinoma was conducted on rat model and the dose used may not represent the clinical therapeutic dose. Further investigation is needed in understanding the molecular mechanism and studies on toxicity also essential to develop *Boerhavia diffusa* as herbal drug.

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