Evaluation of anti-cancer activity of apigenin enriched *chamomile* on diethyl nitrosamine and phenobarbital induced hepatocellular carcinoma in rat model

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Abstract

Hepatocellular carcinoma (HCC) is the main liver cancer and mostly affects those who have cirrhosis and chronic liver disease. Hepatocellular carcinoma often develops in patients with long-term liver conditions like cirrhosis due to hepatitis B or C infection, and Matricaria chamomilla flowers have numerous therapeutic activities. Our study focuses to find out the anti-cancer activity of ethanolic extract of Apigenin enriched chamomile (AEC) in diethylnitrosamine and phenobarbitone induced Hepatocellular carcinoma in rat model. Hypothesis of the study was AEC has flavonoids, terpenoids, glycosides which reduce the elevated oxidative stress act as antioxidant. Apigenin is a typical dietary flavonoid that can be found in Chamomile. Chamomile has several physiological uses including antiviral, antibacterial, and antioxidant, anti-inflammatory activities and blood pressure reduction. In-vitro anti-cancer study using MTT assay confirms whether AEC inhibits cancer or not. In in-vivo study diethylnitrosamine was administered (200mg/kg) by single i.p injection and phenobarbitone was administered (0.5ml/kg) p.o for 12 weeks daily. After induction of cancer, rats were treated with AEC (100 and 200mg/kg/b.w) p.o for 98days and animal weight was monitored every week. After the treatment period, SGOT, SGPT, ALP, Bilirubin, LPO level was decreased and body weight, RBC, WBC, total haemoglobin, lymphocytes, monocytes, total protein levels, CAT, GSH, GPx were increased and histopathological findings of liver sections show anti-cancer effect of AEC with normal hepatocytes. When compared to the usual medication 5-fluorouracil, AEC

was assessed for its ability to prevent liver cancer caused by diethylnitrosamine and phenobarbitone. The result indicates that AEC possess beneficial therapeutic activity against HCC.

Keywords: Hepatocellular Carcinoma (HCC), Apigenin Enriched Chamomile (AEC), Serum Glutamic-Oxaloacetic Transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), Alkaline Phosphatase (ALP), Lipid Peroxidation (LPO)

1. INTRODUCTION

Liver disease is a leading cause of death and chronic illness, causing approximately 2 million fatalities annually. It is primarily caused by complications related to cirrhosis, viral hepatitis, and hepatocellular carcinoma. The burden is highest in regions with high rates of hepatitis infections and alcohol use, with non-alcoholic fatty liver disease (NAFLD) becoming an increasingly common cause of liver-related morbidity in many high-income countries [1]. Liver cancer is the 16th leading cause of death worldwide, while cirrhosis is the 11th most prevalent, accounting for 3.5% of all deaths. Alcohol use is prevalent globally, with up to 75 million diagnosed with alcohol-use disorders [2]. Acute hepatitis is primarily caused by drug-induced liver injury, while viral hepatitis remains prevalent. Liver cancer accounts for 810,000 fatalities worldwide, ranking it as the second most prevalent malignant cause of absolute years of life lost and the fourth most frequent reason for cancer-related fatalities [3]. Hepatitis B virus (HBV), hepatitis C virus (HCV), and metabolic disorders are the major causes of it [4]. The use of antiviral medications is reducing the historical dominance of HBV and HCV as primary causes of HCC, but the prevalence of HCC linked to metabolic liver disease is increasing, gradually replacing HBV and HCV as the primary cause [5]. The liver, the largest detoxifying organ, converts toxic chemicals and endogenous metabolites into highly soluble or non-toxic substances that are easier to excrete through urine or bile [6]. Aflatoxin exposure, hepatitis B and C virus infection, liver cirrhosis, environmental contaminants such nitrosamines, and obesity are the main causes of HCC cases linked to primary infections [7]. Local ablation or liver transplantation are potentially helpful alternatives for HCC when the tumor is limited to the liver. However, these treatments are limited by the patient's condition and liver illness in addition to the tumor's size [8]. Recently, the public's interest in several natural substances and herbs has increased due to their strong therapeutic effects as complementary and alternative medicine [9]. A prevalent dietary

flavonoid in chamomile, apigenin, has several physiological properties, including blood pressure lowering, antiviral, antibacterial, antioxidant, and anti-inflammatory effects. In several cancer cell types, apigenin alone can cause mitochondria-dependent apoptosis. According to experimental research, apigenin effectively suppresses several cancer cell lines in both *in-vitro* and *in-vivo* settings [10,11,12]. Diethylnitrosamine (DEN), a strong hepatocarcinogen, is mostly found in smoking, tobacco, food, drinks, cosmetics, industrial pollution, and agricultural pesticides. These are the main risk factors for liver illnesses, and when they develop endogenously, they can lead to a variety of malignancies that are harmful to people's health [13]. DEN is classified as a human carcinogen due to the overwhelming evidence of its carcinogenicity in experimental animal models. It is known to interfere with the nuclear enzymes' ability to replicate and repair DNA by changing their conformation or function. The metabolic biotransformation of nitrosamines produces reactive oxygen species (ROS), which increase gene expression, intracellular signalling pathways, and biochemical processes, ultimately leading to cancer [14]. The development of a trustworthy tumor alternative therapy that results in the least amount of damage on healthy cells is one of the most significant applications in the rapidly evolving field of cancer treatment. As a result, the identification of new compounds originating from plants has been demonstrated to be a chemotherapeutic agent. The bioactive chemical therefore presents a better opportunity to improve the existing standard of care for HCC and other cancers. As a result, the bioactive chemical offers a greater chance to raise the current standard of care for HCC and other malignancies. This study aimed to investigate the anti-cancer effects of apigenin-enriched chamomile against DEN-induced and phenobarbital promoted hepatocellular carcinoma in rat model [15,16].

2. MATERIALS AND METHODS

Drugs and chemicals

Ethanolic extract of Apigenin enriched chamomile was purchased from Herbo nutra, Uttar Pradesh. Diethyl nitrosamine and phenobarbital was purchased from sigma-aldrich.

Cell viability study by MTT assay

Cell lines and culture medium

ATCC provided the HEP-G2 cell lines. In a humidified setting with 5% CO2, the stock cells were cultivated in DMEM/F12 supplemented with 10% inactivated fetal bovine

serum (FBS), 100 IU/ml of penicillin, and 100 µg/ml of streptomycin at 37°C until confluent. The cell was separated using a cell dissociating solution, which contained 0.2% trypsin, 0.02% EDTA, and 0.05% glucose in PBS. The viability of the cells is assessed after centrifugation. Furthermore, a 96-well plate was seeded with 50,000 Jurkat cells per well, and the cells were maintained for 24 hours at 37°C with 5% CO2 [17].

Procedure

The monolayer cell culture was trypsinized, and the corresponding media containing 10% FBS were used to adjust the cell count to 1.0 x 105 cells/ml. 100 microliters of the diluted cell suspension (50,000 cells/well) was put to each well of the 96-well microliter plate. The partial monolayer was rinsed once with medium, the supernatant was removed after 24 hours, and 100 µl of different test drug dosages were added to the partial monolayer in microliter plates. The plates were then incubated for 24 hours at 37°C in an atmosphere with 5% CO2. After incubation, each well received 100µl of MTT (5 mg/10 ml of MTT in PBS), and the test solutions within the wells were disposed. For four hours, the plates were incubated at 37°C with 5% CO2. After removing the supernatant and adding 100 µl of DMSO, the plates were gently shaken to dissolve the formazan. The absorbance was measured using a microplate reader set to 590 nm. The percentage growth inhibition was calculated using the following formula, and the concentration of test medicine needed to inhibit cell growth by 50% (IC50) values were obtained using the dose-response curves for each cell line.

Animal models and experimental design

The Wistar albino rats are 150–200 grams in weight. The animals were kept in controlled environments with a 12-hour light/dark cycle and a temperature range of 20 to 250 degrees Celsius. Throughout the trial, each rat was given a commercial food pellet and unlimited access to fresh water; weight gain was recorded every week. Every animal procedure was carried out with the ethical committee's consent and in compliance with guidelines for the responsible handling and care of lab animals. The Institutional Animal Ethical Committee (IAEC) KMCRET/ReRc/MPharm/88/2024 for Animal Care approved the study's protocols, which were in compliance with the Government of India's Committee for the Control and Supervision of Experiments on Animals (CCSEA) guidelines. The methods used in the study

were also carried out in compliance with the approved guidelines. The Biogen Laboratory Animal Facility in Bangalore provided the animals (Reg. No: 971/bc/06-CCSEA). The rats were split into five groups at random, with six animals (n=8) in each group.

Preparation of DEN and PB

The animals were weighed individually before drug induction The appropriate dosage for each animal was determined based on its body weight. DEN (200mg/kg) is prepared using normal saline (0.9%w/v) as a solvent. PB was prepared using distilled water as solvent. The PB (0.05%) was dissolved in distilled water completely without any aggregates; sonicated to remove air bubbles [18]

Group-1: Control

Rats were treated with 0.9% w/v of normal saline throughout the experiment

Group-2: Negative Control

DEN (200mg/kg, *i.p*), single induction+ Phenobarbital (0.5ml/kg, *p.o*)

Group-3: Standard

DEN (200mg/kg, *i.p*), single induction+ Phenobarbital (0.5ml /kg, *p.o*) + rats treated with 5-flurouracil (25 mg/kg, *i.p*)

Group-4: Test 1

DEN (200mg/kg, *i.p*), single induction+ Phenobarbital (0.5ml /kg, *p.o*) + rats treated with AEC extract (100mg/kg, *p.o*)

Group-5: Test 2

DEN (200mg/kg, *i.p*), single induction+ Phenobarbital (0.5ml /kg, *p.o*) + rats treated with AEC extract (200mg/kg, *p.o*)

Collection of blood

Blood was drawn using deep inhalation anesthesia using ketamine at the conclusion of the treatment period. Retroorbital punctures were used to obtain blood samples, which were then

allowed to clot for an hour at room temperature. Separate the serum by centrifuging it for 10 minutes at 10,000 rpm, then examine it for the following findings.

Estimation of haematological parameters

Following the collection of blood samples from the retroorbital sinus, the outcomes were examined for haematological parameters (RBC, WBC, Haemoglobin, polymorphs, lymphocytes and monocytes) [19].

Estimation of serum biochemical parameters

Serum was separated and levels of Serum glutamate pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), Alpha-feto protein (AFP), urea, creatinine [20].

Estimation of oxidative stress biomarkers

Levels of enzymatic and non-enzymatic antioxidants, such as SOD, CAT, GSH, and GPX, were measured in liver tissue. Liver tissues are used to test LPO as well [21].

Histopathology Analysis

Hematoxylin and eosin staining was utilized to evaluate the histological analysis, and paraffin-embedded fixed segments were stained with formalin 10% to identify hepatocellular carcinoma.

Statistical Analysis

Data were expressed as mean ± SD and one-way ANOVA followed by Dunnett's test multiple comparison test. The obtained data were analyzed using Graph Pad Prism 5.0

3. RESULTS

Cell viability study by MTT assay

Table 1. Effect of AEC extract in the MTT assay on the HepG2 cell line

Concentration (µg/ml)	Cytotoxicity (%)	Cell Viability (%)	Cytotoxic reactivity	IC50 Value
0	0	100	**None**	**51.81 µM**
15	26.25	73.75	**Slight**	
25	40.3607	59.6393	**Mild**	
50	57.8474	42.1526	**Moderate**	
75	66.9827	33.0173	**Moderate**	
100	76.2411	23.7589	**Severe**	

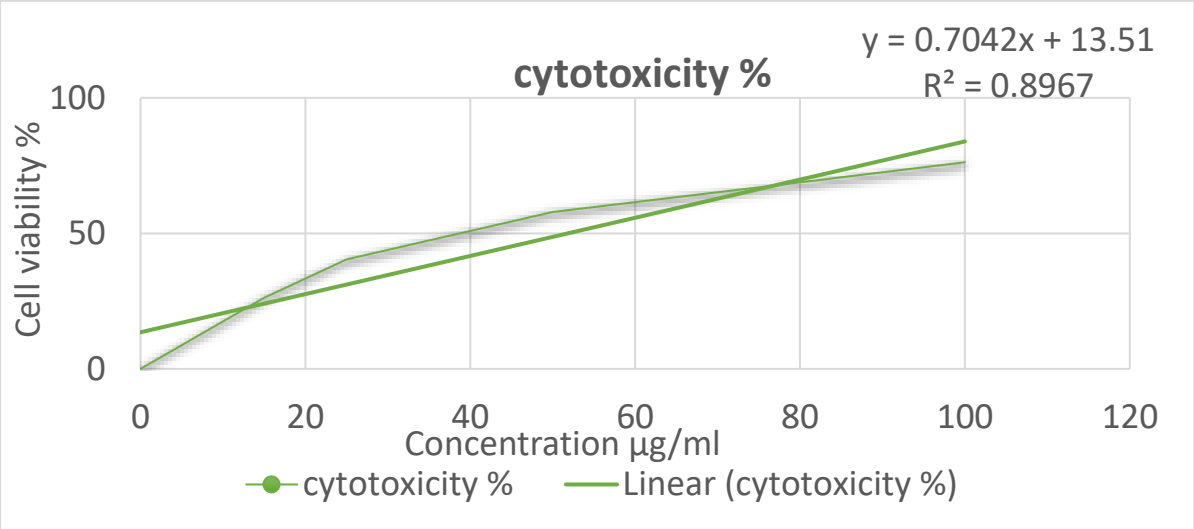
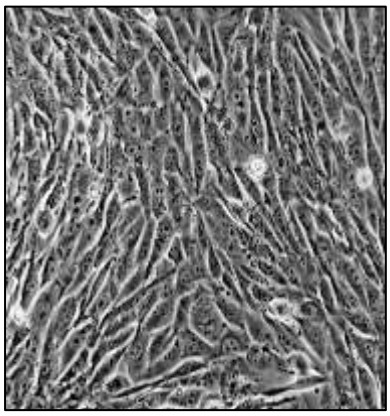


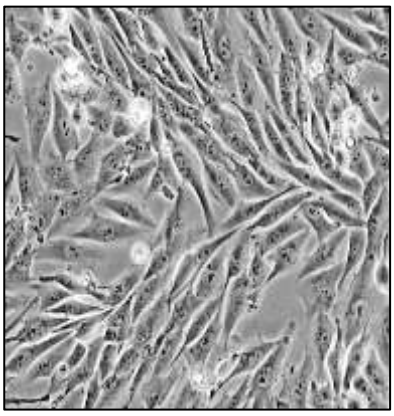
Figure 1: Effect of AEC extract on HepG2 cell line in MTT assay

In MTT assay various concentrations of ethanolic extract of Apigenin enriched chamomile (AEC) (0-100µM) on HepG2 Cell line and the percentage of Cell Viability was studied. The IC_{50} Value of (AEC) on MTT assay was found to be 51.81µM (fig 1)

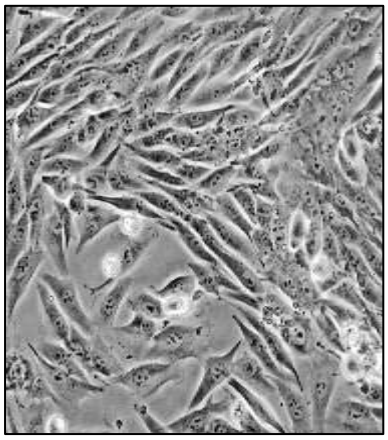
Effect of AEC on cell viability



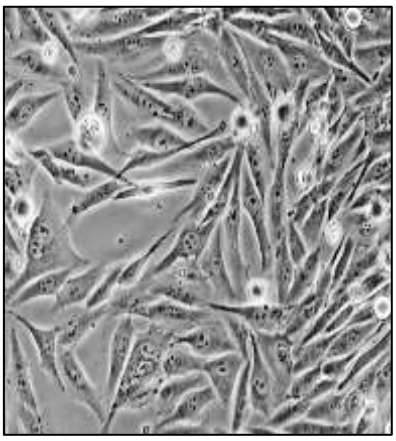
HepG2 Cell line - CONTROL



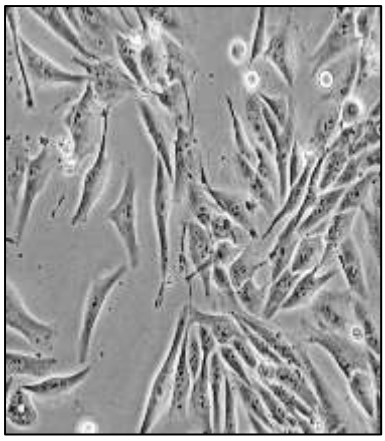
HepG2 Cell line - (15 µg/ml AEC)



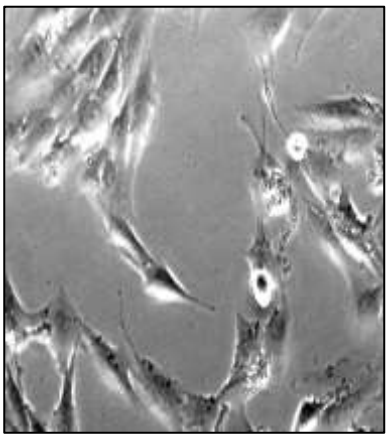
HepG2 Cell line - (25 µg/ml AEC)



HepG2 Cell line - (50 µg/ml AEC)



HepG2 Cell line - (75 µg/ml AEC)



HepG2 Cell line - (100 µg/ml AEC)

Figure 2: Effect of AEC on cell viability (HepG2 cell)

Effect on body weight

The experimental animals' starting and ending body weights were measured. When compared to initial body weight, the weight of the rat decreased after induction with DEN and promoter phenobarbital (Figs 3 and 4).

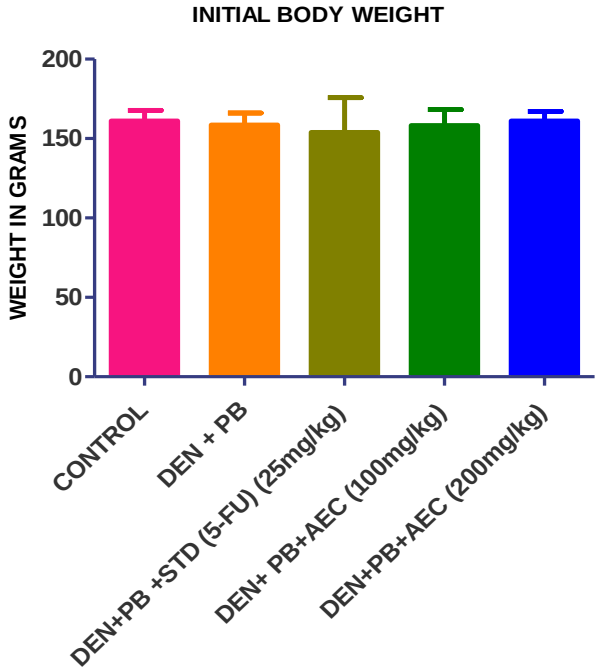


Figure 3: Initial body weight

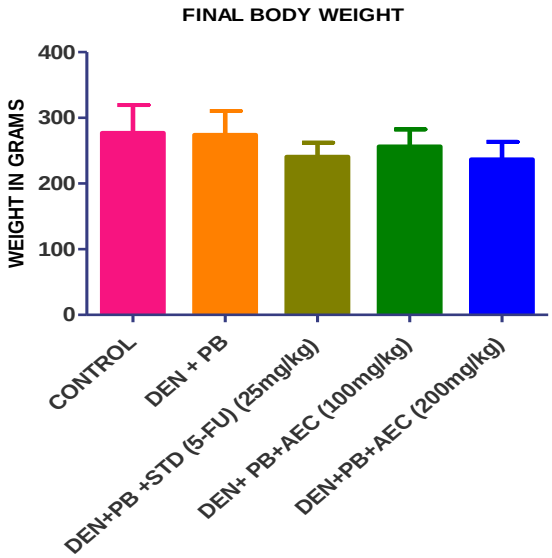


Figure 4: Final body weight

Liver weight analysis

The weight of the liver significantly increases in the illness group and significantly decreases in the AEC-treated group (Fig. 5).

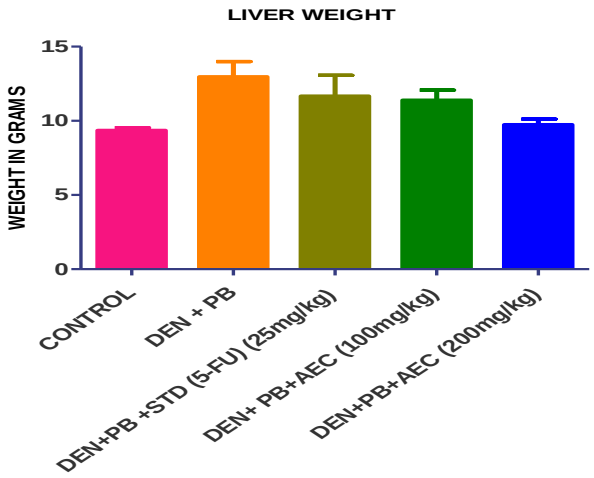


Figure 5: Liver weight analysis

Tumor analysis

The negative control shows a higher incidence of tumors present in the liver, and after 98 days of treatment, Groups III, IV, V Show a reduced incidence as compared to the control group (Figs. 6 and 7).

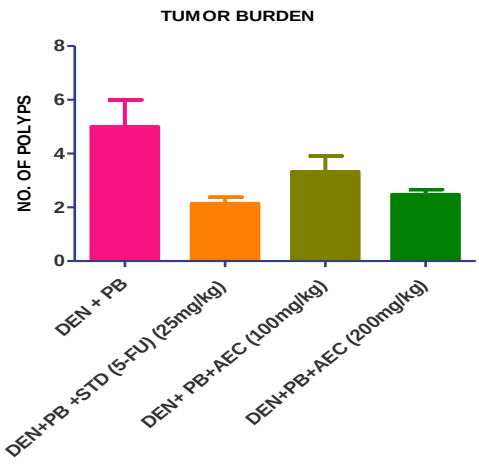


Figure 6: Tumor burden

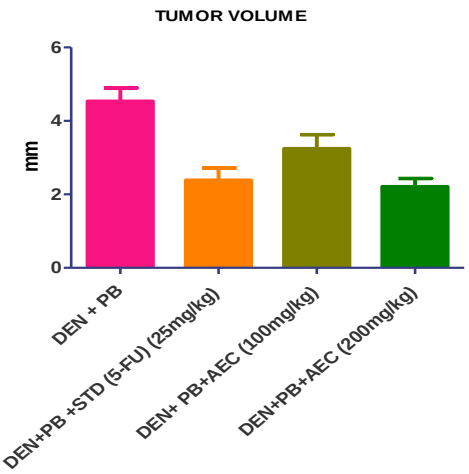


Figure 7: Tumor volume

Effect on Haematological parameters

The disease group shows a significant reduction in RBC, WBC, total Haemoglobin, polymorphs, monocyte, lymphocytes compared to the AEC treated groups (Figs. 8, 9, 10, 11, 12, 13)

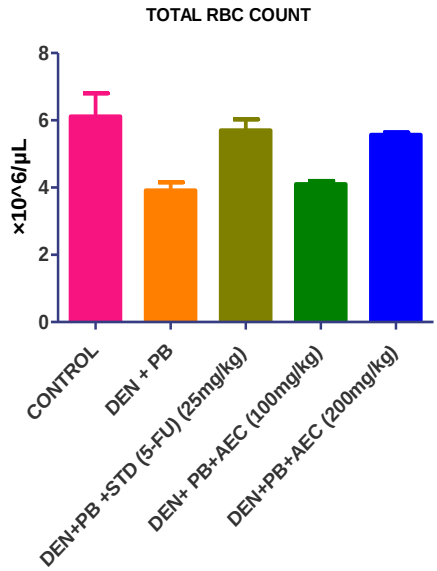


Figure 8: Total RBC count

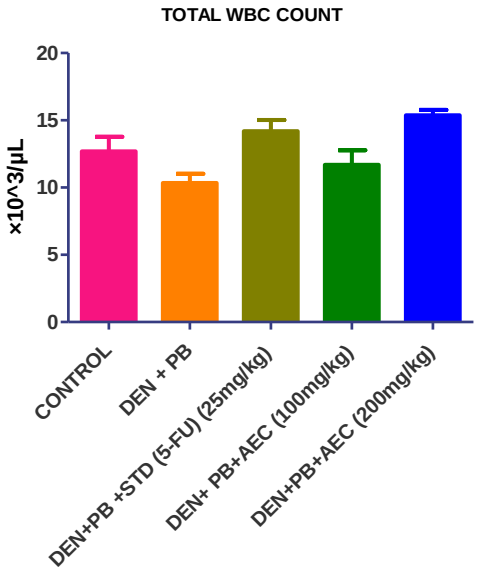


Figure 9: Total WBC count

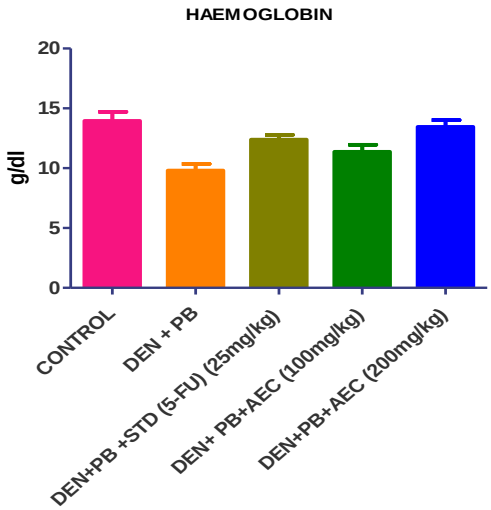


Figure 10: Haemoglobin

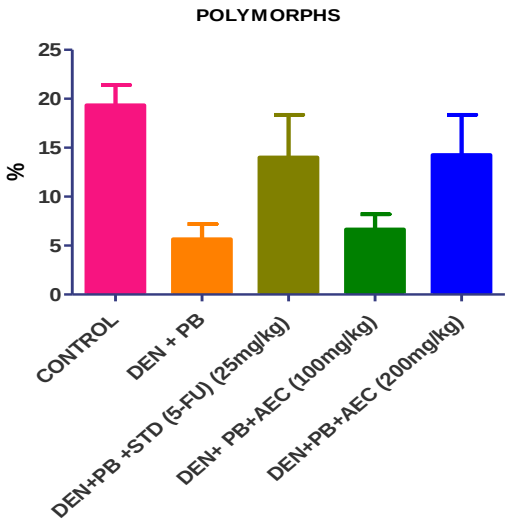


Figure 11: Polymorphs

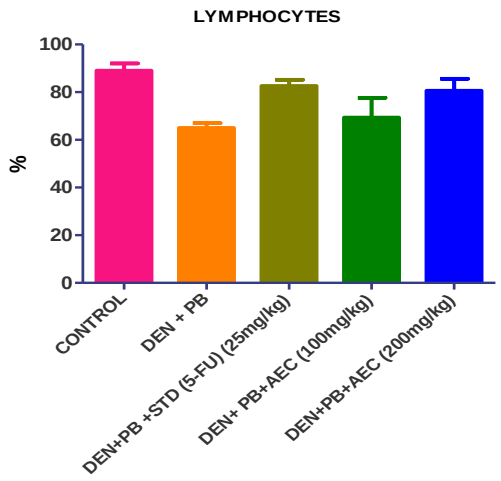


Figure 12: Lymphocytes

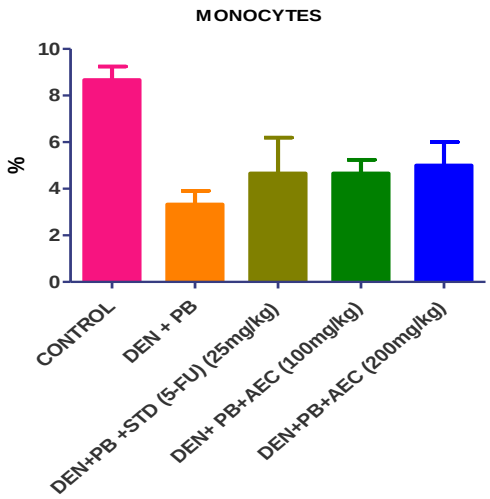


Figure 13: Monocytes

Effect on serum biochemical parameters

Serum Biochemical parameters show altered patterns in hepatocellular carcinoma. DEN promotes the activities of SGOT, SGPT, ALP, and bilirubin (Figs.14, 15, 16, 17) in cancer conditions. The treatment group shows a significant reduction in the level of SGOT, SGPT, ALP, and bilirubin. Tumor markers like LDH and AFP (Figs. 18, 19) show a significant increase in diseased condition while the treated group shows a significant reduction level. DEN increases the activity of urea and creatinine (Figs. 20, 21) in cancer conditions while the treated group shows a significant reduction level.

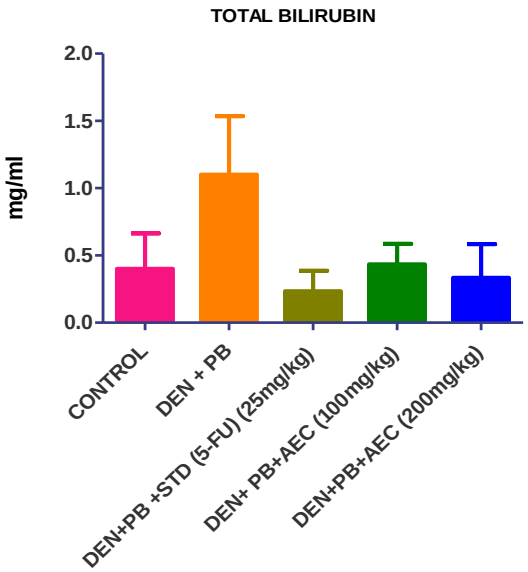


Figure 14: Total Bilirubin

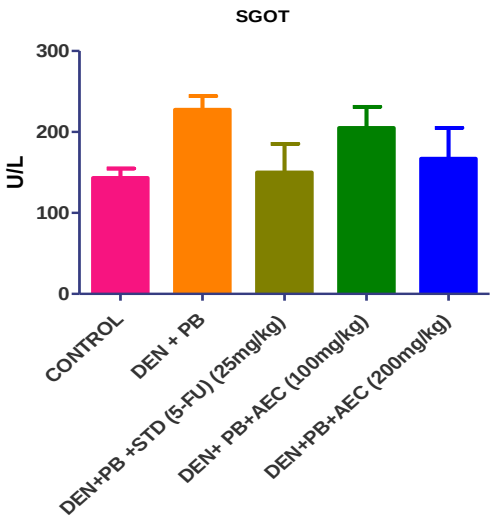


Figure 15: SGO

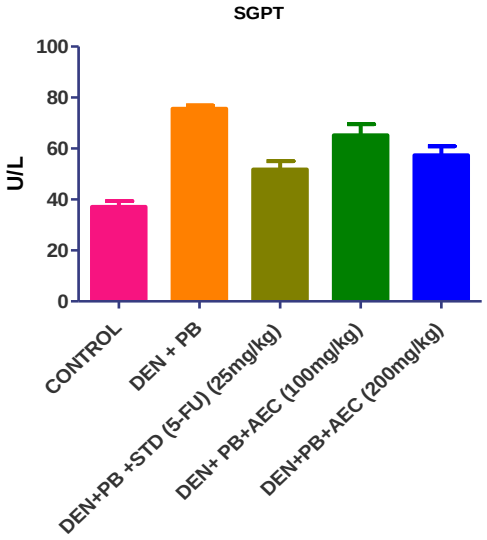


Figure 16: SGPT

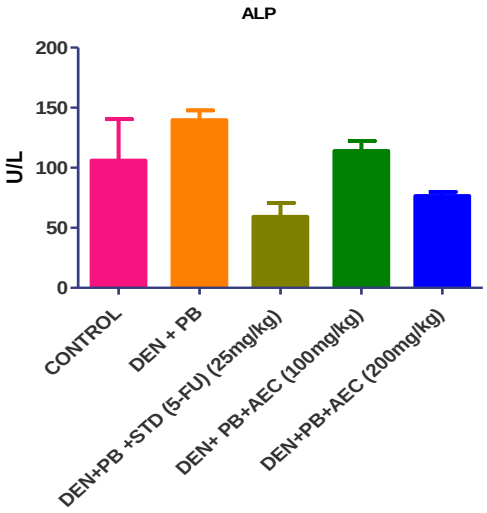


Figure 17: ALP

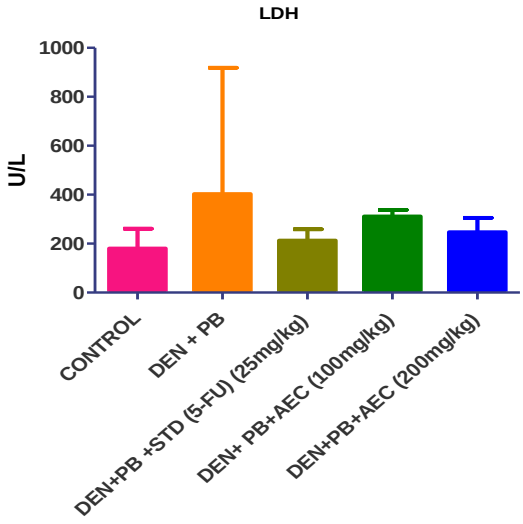


Figure 18: LDH

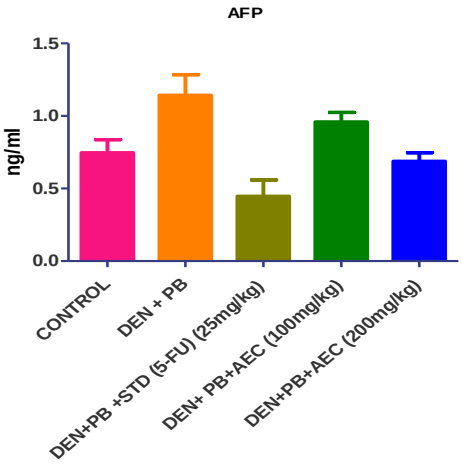


Figure 19: AFP

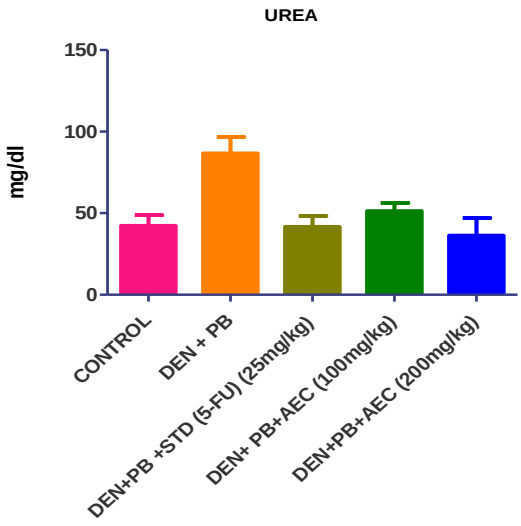


Figure 20: Urea

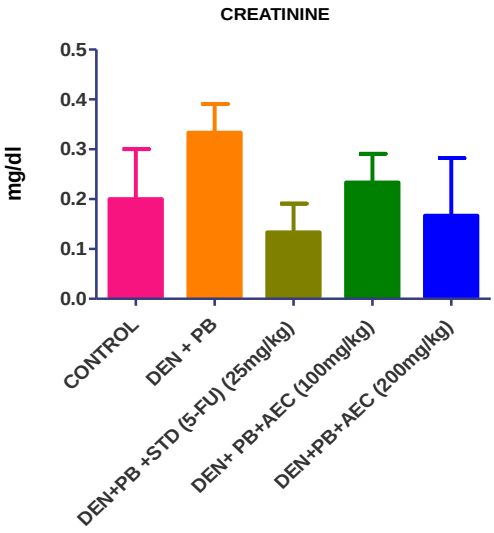


Figure 21: Creatinine

Effect on different antioxidant studies, both enzymatic and non-enzymatic

The disease group significantly increases the level of LPO (Fig. 27) and it reduces the level of SOD, GSH, GPx, and CAT (Figs. 23, 24, 25, 26) in the liver. The treatment groups significantly decrease the level of LPO, and it significantly increase the level of CAT, GSH, and GPx in the liver. Protein and its synthesis were an important phenomenon as well as in hepatocellular carcinoma conditions. DEN declines the level of total protein (Fig. 22) and the treatment group promotes the level of total protein in serum.

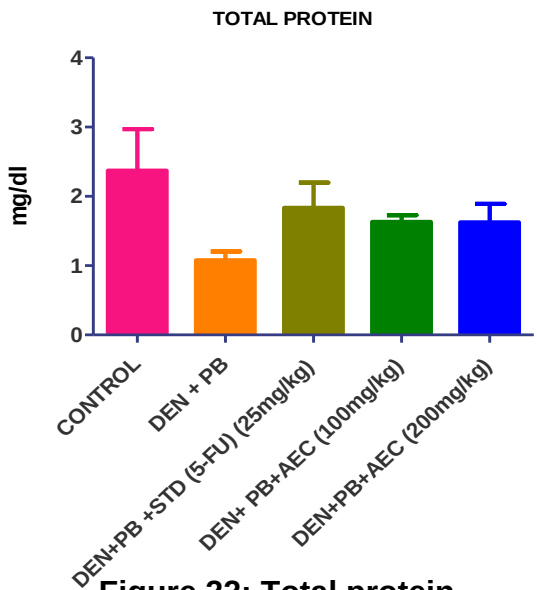


Figure 22: Total protein

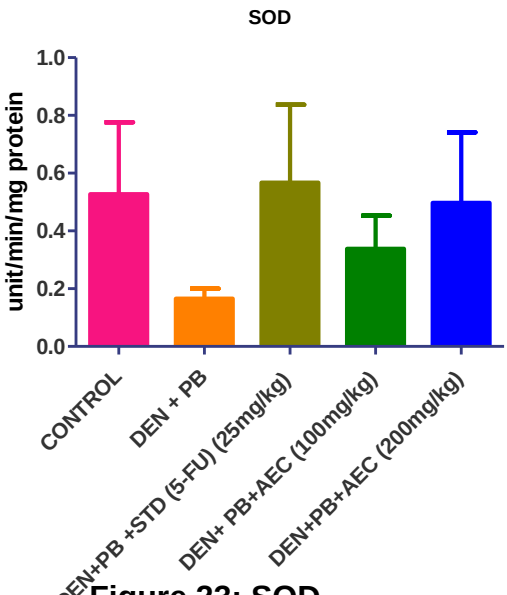


Figure 23: SOD

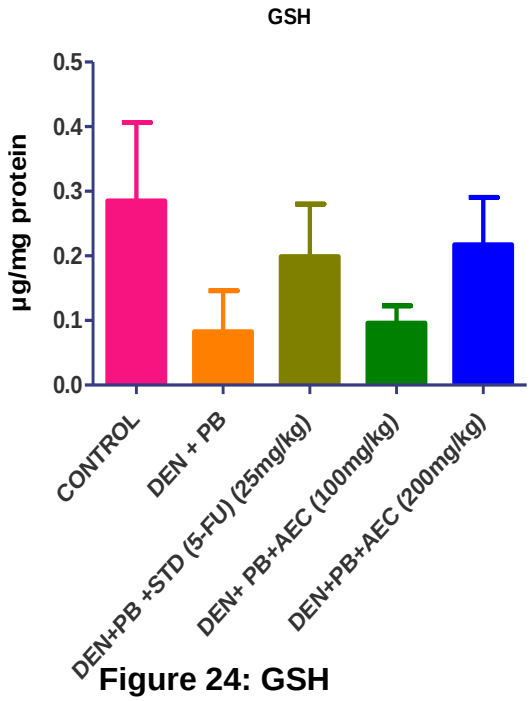


Figure 24: GSH

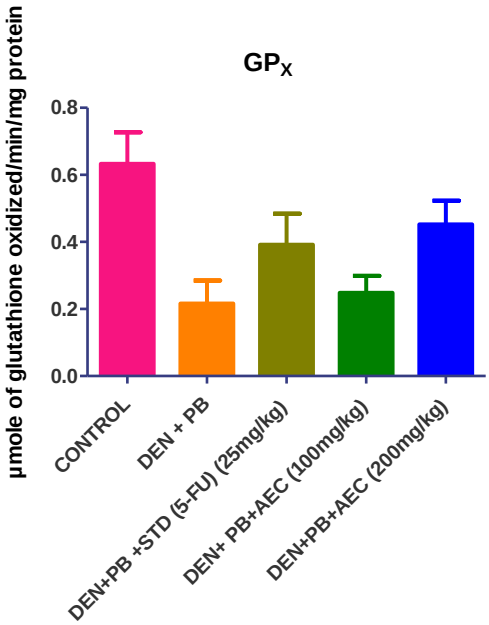


Figure 25: GPX

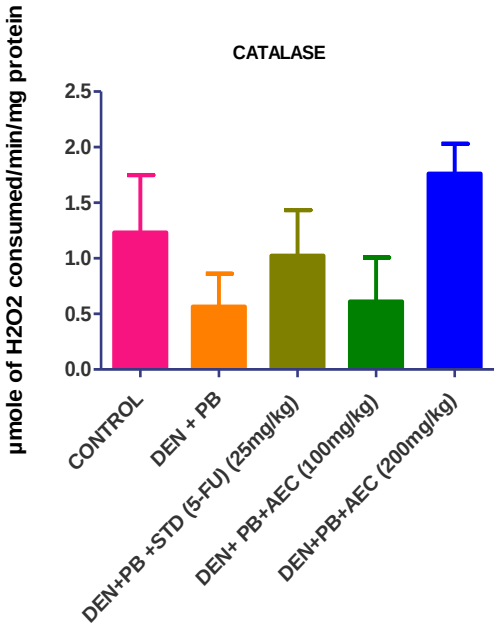


Figure 26: Catalase

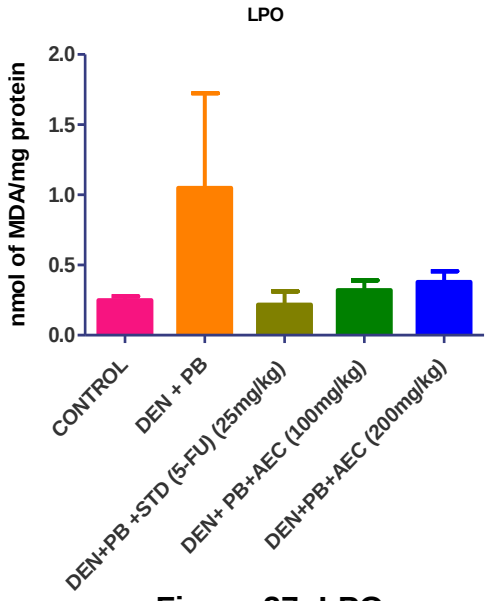
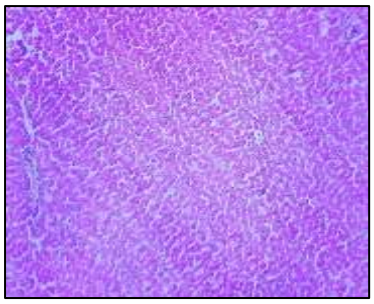
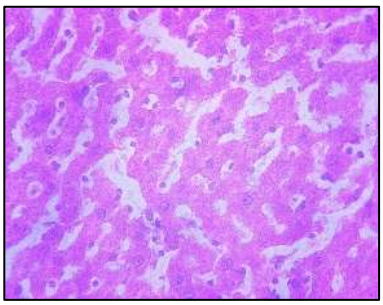


Figure 27: LPO

Histopathological analysis

Group 1: Control





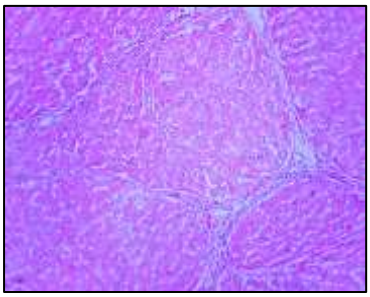
10x shows normal lobular structure 40x displays healthy hepatocytes

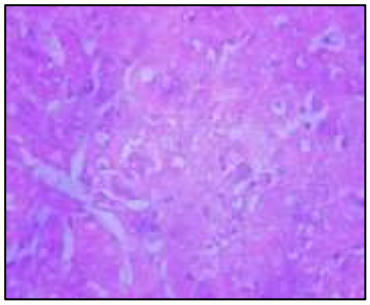
Figure 28(a)

The control liver shows mild altered architecture; hepatocytes are arranged in cords and strands with interface hepatitis. Central vein mild. Congestion. Portal triad shows mild periportal inflammation. Sinusoids show normal

Group II: DEN+PB

(Disease control)



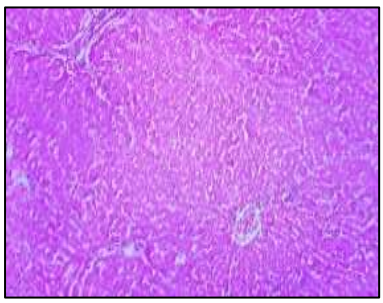


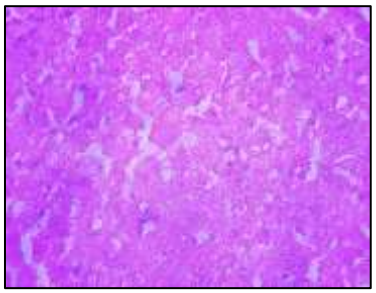
10x shows infiltrating neoplasm 40x shows infiltrating neoplasm

Figure 28(b)

The disease-controlled liver exhibits a malignant tumor made up of lobules of cells. Individual cells are large and pleomorphic round to oval with abundant eosinophilic cytoplasm and large nuclei with prominent nucleoli. Pyknotic debris is seen. 2-3 mitosis/hpf.

Group III: DEN+PB+ 5-FLUROURACIL (25 mg/kg)



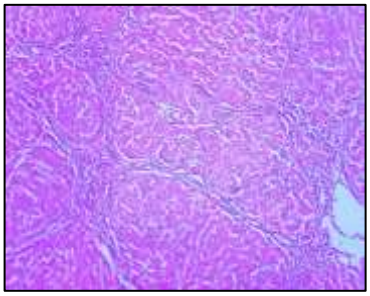


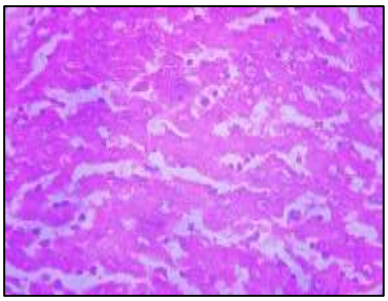
10x shows residual neoplasm 40x shows residual neoplasm

Figure 28(c)

DEN+PB+5-Fluorouracil (25 mg/kg)-treated liver shows liver show altered architecture with a focal area showing residual neoplasm. Individual cells are round to oval, big, pleomorphic, and have large, prominent nuclei and a lot of eosinophilic cytoplasm.1-2 mitoses / hpf seen.

Group IV: DEN+PB+ AEC LOW DOSE (100 mg/kg)



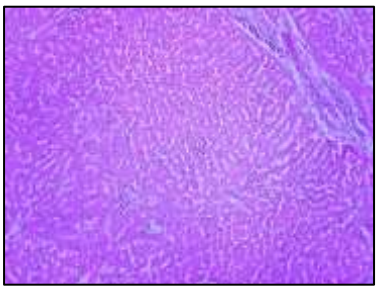


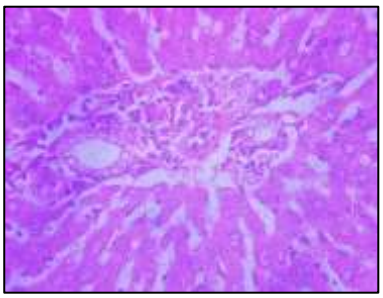
10x shows stroma with inflammatory cells 40x shows oval cells with vesicular nuclei

Figure 28(d)

DEN+PB+AEC Low Dose (100 mg/kg) treated liver shows a malignant neoplasm composed of cells arranged in lobules. Individual cells are large and pleomorphic, round to oval, with abundant eosinophilic cytoplasm and large nuclei with prominent nucleoli. Pyknotic debris is seen. 2-3 mitosis/hpf. Surrounding area shows normal liver parenchyma with dense inflammatory infiltrates.

GROUP V: DEN+PB+ AEC HIGH DOSE (200 mg/kg)





10x shows normal lobular structure 40x displays healthy hepatocytes

Figure 28(e)

DEN+PB+AEC High-dose (100 mg/kg) treated livers show mildly altered architecture; hepatocytes are arranged in cords and strands with interface hepatitis. Central vein and sinusoids show mild dilatation. Portal triad shows mild periportal inflammation.

4. DISCUSSION

Every year, liver disease causes two million fatalities worldwide, with one million resulting from complications from liver cirrhosis, one million from viral hepatitis, and one million from hepatocellular carcinoma. Hepatocellular carcinoma (HCC), the most common subtype of cancer, accounts for over 90% of all cancer cases. As the third most common cause of cancer-related deaths worldwide in 2020, liver cancer emerged as a significant global health concern, following colorectal and lung cancer [22].

Hepatocellular carcinoma (HCC) is the most common and lethal kind of cancer in the world. Patients with significant liver disease are most commonly affected by this aggressive, rapidly developing cancer. More than 80% of people with HCC have cirrhosis from other liver diseases, which is a significant risk factor for the development of this cancer. Other known causes of hepatocellular carcinoma include hemochromatosis, immunological disorders, and metabolic abnormalities, including α1-antitrypsin deficiency. Because men are two to three times more likely than women to be impacted by HCC, there is a significant gender disparity that drives up incidence and death rates globally [23].

In experimental animal models, diethylnitrosamine (DEN), often referred to as N-nitrosodiethylamine, is frequently utilized as a carcinogen. In several animal species, DEN produces acute hepatotoxins and carcinogens. High doses of DEN given parenterally or through prolonged oral feeding can successfully develop hepatic tumors in animals. Animals given DEN develop a variety of cancers, including those of the liver, gastrointestinal system, skin, respiratory system, and hematopoietic cells. The cytochrome P450 (CYP) enzymes in the liver must biotransform DEN because it is not a direct carcinogen. This biotransformation results in DNA-adduct formation through an alkylation mechanism. The activity of hepatic demethylase is increased by phenobarbitone. Administration of phenobarbitone in the drinking water causes a steady and prolonged rise in the amount of cytochrome P450 in rat liver [24].

Here, 5-fluorouracil (5-FU) acts as a standard drug that is crucial to the treatment of cancer by decreasing or preventing the proliferation of cancer cells. 5-FU is an antimetabolite medication that stops cancer cells from producing proteins and DNA by mimicking a DNA and RNA building block. 5-Flurouracil is having an effective anticancer therapy as suggested by experimental and clinical studies [25].

In this study we used ethanolic extract of apigenin-enriched chamomile as the test drug. Ethanolic extract of AEC (apigenin-enriched chamomile) may have a significant anti-cancer effect due to the presence of flavonoids. Apigenin is a common dietary flavonoid found in Chamomile has multiple physiological functions, such as antiviral, antibacterial, antioxidant, and anti-inflammatory activities and blood pressure reduction. Hence, this study aimed to evaluate the anticancer activity of ethanolic extract of apigenin-enriched chamomile on hepatocellular carcinoma in Wistar rats. Parameters used to evaluate the anti-cancer activity of ethanolic extract of AEC are tumor weight and tumor burden and haematological and biochemical parameters [26].

Based on study report, apigenin-enriched chamomile in different concentrations shows inhibition of the viable cells of HepG2 that shows AEC inhibits hepatocellular carcinoma. In tumor analysis negative control shows a higher incidence of tumor present in the liver, and after 98 days of treatment, Group III, IV, V Shows a reduced incidence as compared to control group. Compared to those treated with AEC, the disease group has a marked decrease in RBC, WBC, total hemoglobin, polymorphs, monocytes, and lymphocytes. The body weight of the disease group significantly decreased, while the body weight of the

AEC-treated group significantly increased. The weight of the liver significantly increases in the disease group and significantly decreases in the AEC-treated group. Tumor burden (number of polyps) and tumor volume significantly rise in the disease group, whereas both significantly decrease in the AEC-treated group. Serum Biochemical parameters show altered patterns in hepatocellular carcinoma. In cancerous situations, DEN stimulates the functions of bilirubin, SGOT, SGPT, and ALP. The levels of SGOT, SGPT, ALP, and bilirubin are significantly lower in the treatment group. Tumor markers like AFP and LDH show a significant increase in diseased conditions while the treated group shows significant reduction level. Lipid peroxidation (LPO) is markedly elevated in the disease group, while CAT, GSH, and GPx levels in the liver are decreased. Treatment groups considerably raise the levels of CAT, GSH, and GPx in the liver while significantly lowering the levels of lipid peroxidation (LPO). Protein and its synthesis were important phenomena as well as in hepatocellular carcinoma conditions. The total protein level in serum is raised by the treatment group, whereas it is decreased by DEN.

Histopathological studies:

The disease group exhibits interface hepatitis and malignant neoplasms made up of rearranged cells with altered lobular architecture. Vacuolation of the cytoplasm in individual hepatocytes. The portal triad shows bile duct hypertrophy. The central vein is dilated. Sinusoids exhibit congestion and dilatation. Lobular architecture is normal in the treatment group. Hepatocytes on their own appear normal. Periportal inflammation is shown by the portal triad. Congestion is seen in the central vein. Sinusoids exhibit modest congestion and dilatation.

5. CONCLUSION

Hepatocellular carcinoma (HCC) is the main liver cancer and mostly affects those who have cirrhosis and chronic liver disease. Increased lipid peroxidation and oxidative stress brought on by ROS are the primary characteristics of HCC. It is expected that in the years afterward, the threat posed by HCC will only grow. The present study aimed at evaluating the anti-cancer activity of apigenin-enriched chamomile on DEN and phenobarbital promoted hepatocellular carcinoma in male Wistar rats. The focus of the study was set on the efficacy of the AEC to prevent HCC.

In the present study, findings indicate that, ethanolic extract of apigenin-enriched chamomile (AEC) is effective in inhibiting the DEN and PB induced hepatocellular carcinoma because it has flavonoids, terpenoids and phenolic compounds that suppress the

elevated oxidative stress and lipid peroxidation that act as an antioxidant. *In vitro* anticancer activity of apigenin-enriched chamomile in different concentrations shows inhibition the viable cells of HEP-G2 which shows AEC inhibits liver cancer. From serum biochemical parameters, AEC significantly reduces the elevated levels of SGOT, SGPT, ALP and bilirubin during liver cancer. Major tumor markers like LDH and AFP also decrease in AEC treated groups. *In vivo* antioxidant activity of AEC shows a decrease in the level of lipid peroxidation (LPO), and it significantly increases the level of CAT, GSH and GPx in the liver. In animal studies, it was confirmed by changes observed in histopathological evaluation. Therefore, it can be concluded from the current study that AEC may be able to both prevent and lessen the problems associated with HCC. It appears encouraging that the study's data can be further verified in subsequent research, which could ultimately provide a high level of protection against HCC.

6.CONFLICT OF INTEREST

The authors declare no conflict of interest.

7. REFERENCE

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