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Research Article
Valproate Ameliorates Diethylnitrosamine/Phenobarbital-Induced Hepatic Cancer via the Role of TNF-α and TGF-β1

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Abstract

Background and Objective: Hepatocellular Carcinoma (HCC) constitutes approximately 90% of all primary cases of liver cancer and no appropriate management of pharmacological medications are currently available on the market for diagnosis or cure of this lethal disease. This study aimed to scrutinize the chemoprotective effect of valproate against diethylnitrosamine(DEN) induced HCC in rats. **Materials and Methods:** Single intraperitoneal injection of DEN (200 mg kg^{-1}) was used for induction of the HCC and Phenobarbital (Pb) was used to boost the HCC and rats were treated with Doxorubicin (DOX) and valproate (1.25 and 2.5 mg kg^{-1}). The rats were macroscopically examined and estimated the organ weight. Hepatic, biochemical, antioxidant, pro-inflammatory cytokines and inflammatory mediators were estimated. **Results:** Valproate considerably reduced the hepatic nodules and altered the organ weight as compared to DEN treated rats. valproate considerably reduced the level of α-fetoprotein and other hepatic enzymes (Alkaline Phosphates (ALP), Aspartate Aminotransferase (AST) and Alanine Transaminase (ALT)). Valproate significantly increased the level of total protein, albumin, globulin, A/G ratio and reduced the level of total bilirubin. Valproate considerably reduced the LPO level and boosted the Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Glutathione (GSH), Catalase (CAT), Glutathione-S-Transferase (GST) level. **Conclusion:** Valproate reduced the cytokines such as TNF-α, IL-6, IL-1β and inflammatory mediator include NF-kB. Valproate significantly reduced the TGF-β1 level in hepatic tissue. The finding exhibited the therapeutic effect of valproate for hepatocellular carcinoma treatment.

Key words: Valproate, hepatocellular carcinoma, oxidative stress, inflammation, SOD, albumin, α-fetoprotein, doxorubicin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hepatic Injury-induced via hepatitis viruses, fatty acid dietary, alcohol, carcinogenic pollutants, alcohol and drugs in which inflammation plays a significant role in wound healing of hepatic and could be sturdily associated with fibrosis, cirrhosis and hepatocellular carcinoma expansion[1]. Generally, patients who have cirrhosis or fibrosis have hepatic cancer and the process of inflammatory is the main channel of hepatocarcinogenesis. HCC is the globe's second-largest cause of cancer-associated mortality and approximately 850,000 incidences occur every year[2,3].

HCC accounts for approximately 90% of all primary liver cancer cases and presently no effective management or pharmacological drugs are available in the market to treat or cure this fatal ailment. Infection with chronic Hepatitis B Virus (HBV) makes up more than half of all cases related to HCC[4,5]. More than 275 million populations are estimated to be chronically infected with HBV and chances of developing diseases with a risk of a lifetime around 15-40% involving cirrhosis, liver failure and HCC[3,6]. To develop better treatments, therefore, a better understanding of HCC pathogenesis is required[7].

Several risk factors contribute to HCC development such as viral infection involving the virus associated with hepatitis B and C, exposure to aflatoxin, ingestion of alcohol and iron overload. HCC incident rates in various Asian countries, including Thailand, are rising[8,9]. Most HCC patients have a poor prognosis due to the first advanced-stage detection of disease. There have been numerous different therapies designed to advance the prognosis and patients survival for the long term, Although various limitations continue, severe side effects occur with this all therapy i.e., fatigue, pain, diarrhoea, nausea, vomiting, hair loss and resistance to cancer cell after treatment[8-10]. Recently, traditional medicines from plants were obtained for cancer therapy and a fascinating centre of attraction for the researcher too[3]. This experimental study aims to estimate the chemoprotective effect of valproate against DEN induced HCC in rats and explore the underlying mechanism.

MATERIALS AND METHODS

Study area: The experimental study was carried out in the Department of Pharmacy, Qingdao Hiser Medical Center (SDFAH2018077), China from March-July, 2020.

Chemical: Valproate, diethylnitrosamine and phenobarbital were obtained from Sigma Aldrich (Sigma, California, USA), ALP, AFP, AST, ALP Elisa kits were purchased from the Diasys, Germany. ELISA kit TGF-β1 (Novateinbio Cat. No. FME100129) and TNF-α (Sigma-Aldrich Cat. No. RAB0480). All chemical and reagent used in the experimental research is analytical grade.

***In vivo* activity:** Hepatic cell lines HUH-7 was used for the estimation of cell viability and MTT assay using standard kits.

In vivo protocol

Experimental animal: Swiss Albino Wistar rats (150-175 g gender male) were used in this study. The rats were procured from the departmental animal house. The rats had been housed in normal laboratory conditions such as temperature (21±3°C), humidity (65%) and light cycle (12/12 hrs). The rats were acclimatized for 1 week before the experimentation. All the experimental study was conducted by following the International guidelines. The whole animal experimental study was approved by the Qingdao Hiser Medical Center (SDFAH2018077).

Drug preparation: DEN (200 mg kg^{-1}) suspended in the Phosphate Buffer Solution (PBS) was used for induction hepatic cancer and phenobarbital (8 mg kg^{-1}) was used as the promoter of hepatic cancer (every week)[11,12]. Valproate (test drug) was solubilized in the carboxyl methylcellulose (1%) to prepare a suspension.

Induction and confirmation of HCC: HCC was confirmed in rats by estimation of alpha-fetoprotein (AFP) levels in the rats after 14 days. The rats were randomly selected and having α-FP over 200 ng mL^{-1} was selected for the next study.

Experimental protocol: The rats were randomly divided into the following groups after acclimatization time and each group contains 12 rats:

- Group 1: Normal control rats
- Group 2: DEN/Pb
- Group 3: DOX+DEN/Pb
- Group 4: DEN/Pb+valproate (2.5 mg kg^{-1})
- Group 5: DEN/Pb+valproate (5 mg kg^{-1})

The rats have received the oral administration of a tested drug (once a day) until 20 weeks. All group rats' food intake, water and body weight were measured at regular time

intervals. All group rats were anaesthetized using thiopental sodium at the end of the experimental duration and extracted the blood sample by puncturing the retro-orbital plexus. The blood samples collected were held in tube-containing EDTA and centrifuged for 10 min at 4°C at 15000 g rpm to remove the supernatant. For biochemical calculation the clear supernatant has been stored at -80°C. At the end of the experimental protocol, the rats were sacrificed through dislocation of cervical and quickly removed the hepatic tissue and prepared homogenates for estimation of enzymatic and non-enzymatic parameters.

Hepatic parameters: The serum enzyme activity includes Alkaline Phosphates (ALP), Alpha-Fetoprotein (AFP), Aspartate Aminotransferase (AST) and Alanine Transaminase (ALT) was analyzed using an automated biochemistry analyzer (Hitachi 902; Tokyo, Japan).

Non-hepatic parameters: An automated biochemistry analyser (Hitachi 902; Tokyo, Japan) has been used to estimate non-hepatic parameters such as bilirubin, calcium, creatinine, potassium and sodium[13].

Cellular antioxidant parameters: Oxidative stress was determined via scrutinized the enzymatic and non-enzymatic antioxidants viz., Glutathione Peroxidase (GPx), Glutathione-S-Transferase (GST), Catalase (CAT), Superoxide Dismutase (SOD) and reduced glutathione in the hepatic tissue homogenate.

Glycoproteins and ATPase activity: Glycoprotein includes hexosamine, sialic acid, hexose, fucose and membrane-bound ATPase enzyme activities, especially Ca^{2+}ATPase, Mg^{2+}ATPase and $Na^{+}K^{+}$ATPase were determined in the hepatic tissue homogenate[14].

Cytokines and inflammatory mediators: Cytokines include TNF-α, IL-6, IL-1β and inflammatory mediators viz., NF-κB was estimated via using the ELISA kits via following the manufacture instruction.

TGF-β1: The level of TGF-β1 was estimated in the hepatic tissue of all group rats by following the manufacturing instruction of ELISA kits.

Statistical analysis: The statistical research was scrutinized using GraphPad Prism 5 (St. Louis, USA). All result in triplicate was obtained and presented as Mean±SEM. Dunnett's post-test was used for statistical analysis. Where $p<0.05$ is regarded as significant.

RESULTS

***In vitro* cytotoxic studies:** The data of Fig. 1 illustrates the HUH-7 cell lines percentage growth inhibition at various concentrations of Valproate. MTT assay has been tested for the *in vitro* cytotoxic activity of the Valproate at various concentrations against hepatic cancer cell lines (HUH-7). The activity of cell proliferation reduced when the Valproate concentration increased. For the Valproate, IC_{50} cell inhibition data was established at μg mL^{-1}.

Body weight: The result of Table 1 shows the rats' body weights subject to various treatments. Rats demonstrate constant body weight in the standard control group during the treatment period. DEN induced rats showed a body weight loss, these reductions among the weeks were not significantly different from that. While the supplementation of the Valproate at a high dose level and standard drug DOX shown a significant up-regulation in their body weight. At the end of the experimental period, a group treated with Valproate at a low dose also retained the body weight.

Macroscopic changes in the hepatic tissue: In the control group rat, liver appearance was normal and macroscopically no nodules were noticed (Table 2). All DEN/Pb-induced hepatic cancer groups in rats observed macroscopic hepatic alteration. In DEN+Pb induced hepatic cancer rats, after 28 days of administration showed hepatomegaly and nodules in small size in the liver with a rough surface. Treatment with Valproate and DOX also showed a presence of small nodules on the surface of the liver at the end of the experiment (Table 3).

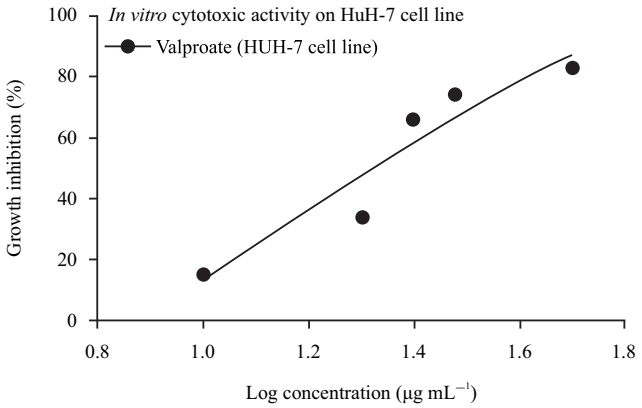

Fig. 1: Cytotoxic activity of valproate on hepatic cancer (HuH-7 cell line)

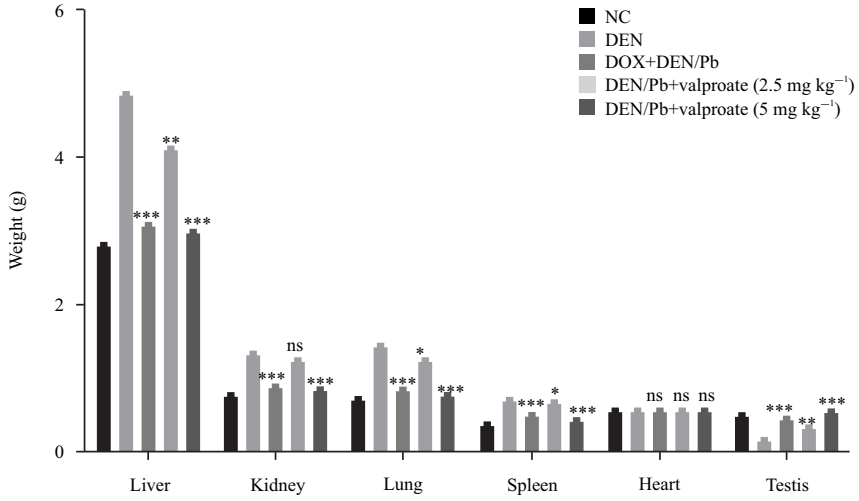

Fig. 2: Effect of valproate on the organ weight of DEN/Pb induced hepatic cancer

NC: Normal control, DEN: Diethylnitrosamine, PB: Phenobarbital, ns: Non-significant. Data represent the Means±SD (n = 6). *p<0.05 is significant, **p<0.01 is more significant and *** p<0.001 is extremely significant

Table 1: Summary of the body weight of the different group of rats

	Body weight (g)			
Groups	Week 1	Week 2	Week 3	Week 4
Control	155.56±4.56	165.4±5.23	178.45±4.93	192.4±5.03
DEN	152±5.23	150.3±6.54	145.9±6.39	138.4±5.94
DOX+DEN/Pb	153.04±4.56^{ns}	160.42±5.03*	168.76±4.23**	178.4±4.56***
DEN/Pb+valproate (2.5 mg kg^{-1})	158.34±5.93^{ns}	161.3±4.89*	166.02±5.67**	171.3±3.45***
DEN/Pb+valproate (5 mg kg^{-1})	156.04±4.45^{ns}	162.7±4.78*	169.54±5.03**	177.45±4.56***

NS: Non-significant. Data represent the Mean±SD (n = 6).*p<0.05, **p<0.01 and ***p<0.001

Table 2: Expansion of hepatic nodules in the DEN induced HCC rats

Groups	Number of rats/number of rats with nodules	Total number of nodules	Tumor incidence (%)
DEN	10/10	201	100
DEN/DOX	10/3	45	30
DEN/Pb+valproate (2.5 mg kg^{-1})	10/6	112	60
DEN/Pb+valproate (5 mg kg^{-1})	10/2	32	20

Table 3: Size distribution and expansion of hepatocyte nodules induced by DEN

			Relative size (% of number size)		
Groups	Total number of nodules	Average number of nodules	≤1 mm	<3 mm>1 mm	≥3 mm
DEN	201	58.32±4.85	92 (45.77)	57 (28.35)	52 (25.87)
DEN/DOX	45	9.45±2.89***	20 (44.44)	15 (33.33)	10 (22.22)
DEN/Pb+valproate (2.5 mg kg^{-1})	112	24.72±2.73***	49 (43.75)	35 (31.25)	28 (25)
DEN/Pb+valproate (5 mg kg^{-1})	32	7.01±1.63***	16 (50)	12 (37.50)	4 (12.50)

NS: Non-significant. Data represent the Mean±SD (n = 6). *p<0.05, **p<0.01 and ***p<0.001

The data of Fig. 2 showed the effect of valproate on the different organs (liver, kidney, lung, spleen, heart and testis) of experimental rats. The data of Fig. 2 exhibited the organ weight of liver (2.8±0.11), kidney (0.75±0.09), lung (0.69±0.08), spleen (0.36±0.06), heart (0.53±0.05) and testis (0.47±0.03), respectively. DEN induced group rats showed the organ weight of liver (4.83±0.25), kidney (1.13±0.12), lung (1.42±0.16), spleen (0.67±0.11), heart (0.54±0.13) and testis (0.14±0.06) and DOX+DEN/Pb group rats showed the organ weight of liver (3.06±0.28), kidney (0.86±0.18), lung (0.83±0.09), spleen (0.47±0.08), heart (0.54±0.06) and testis (0.42±0.02) and valproate (2.5 and 5 mg kg^{-1}) treated group rats demonstrated the organ weight of liver (4.01±0.73 and 2.96±0.17), kidney (1.22±0.23 and 0.84±0.07), lung

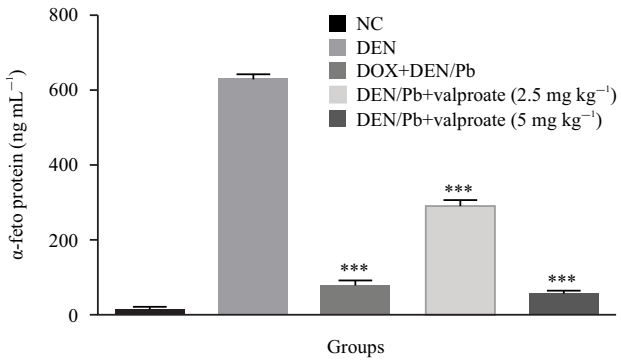

Fig. 3: Summarized the effect of valproate on the α-fetoprotein of DEN/Pb induced hepatic cancer

NC: Normal control, DEN: Diethylnitrosamine, PB: Phenobarbital, NS: Non-significant. Data represent the Means±SD (n = 6).*** p<0.001 is extremely significant

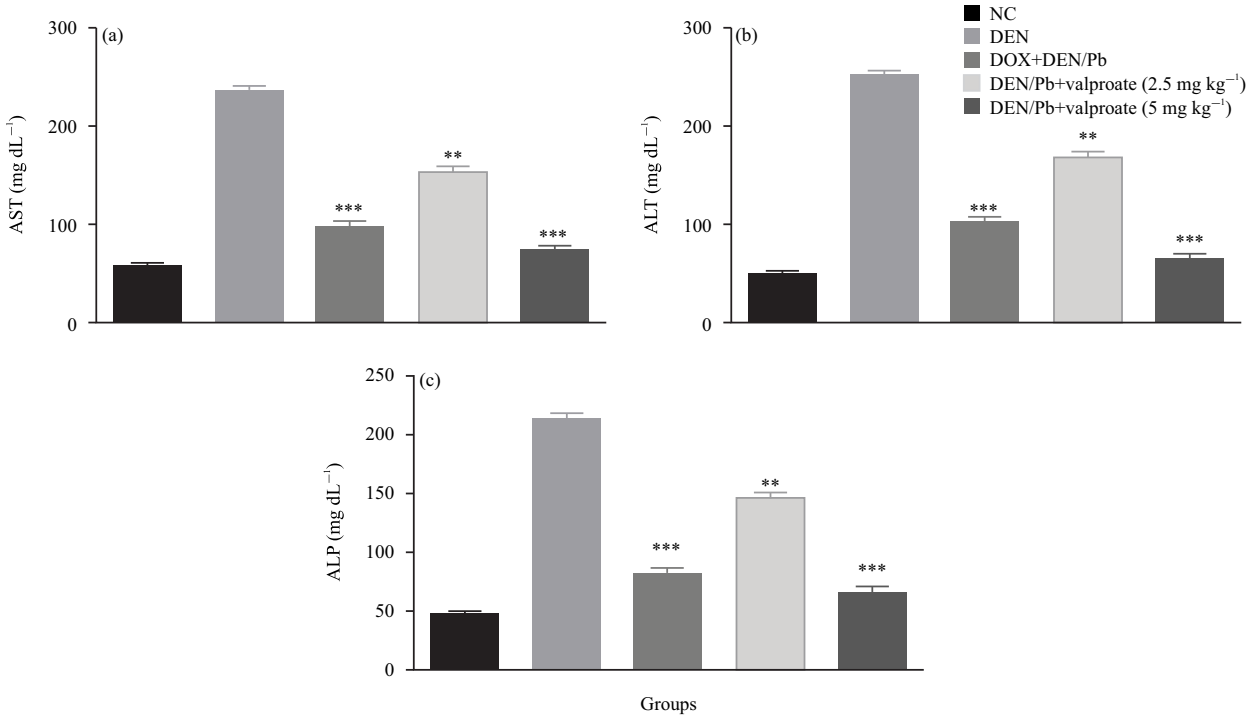

Fig. 4(a-c): Effect of valproate on the hepatic parameter of DEN/Pb induced hepatic cancer

a: AST, b: ALT and c: ALP. NC: Normal control, DEN: Diethylnitrosamine, PB: Phenobarbital, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, NS: Non-significant. Data represent the Mean±SD (n = 6).**p<0.01 is more significant and ***p<0.001 is extremely significant

(1.23±0.34 and 0.75±0.04), spleen (0.65±0.06 and 0.41±0.03), heart (0.52±0.06 and 0.54±0.07) and testis (0.31 and 0.54±0.04), respectively.

Confirmation of the presence of hepatocellular carcinoma: The degree of α-FP was examined after 12 weeks of induction to prove the existence of HCC in the induced rats. Blood was obtained and an α-Fetoprotein assay was performed before cancer was induced with DEN/PB. Before induction, the level of α-FP was normal in the serum of rats. Although, supplementation with DEN and PB cause hepatic cancer in rats and the α-FP level was significantly up-regulated. However, treatment with Valproate at both dose levels down-regulated the level of α-FP in the rats (Fig. 3).

Level of serum liver enzymes in HCC: The data of Fig. 4a-b displayed the AST (Fig. 4a) and ALT (Fig. 4b) activity in the rats. After the induction of DEN+PB, the rats showed enhanced activity of liver enzymes (ALT and AST) relative to those in control rats, indicating the potential obstruction to liver

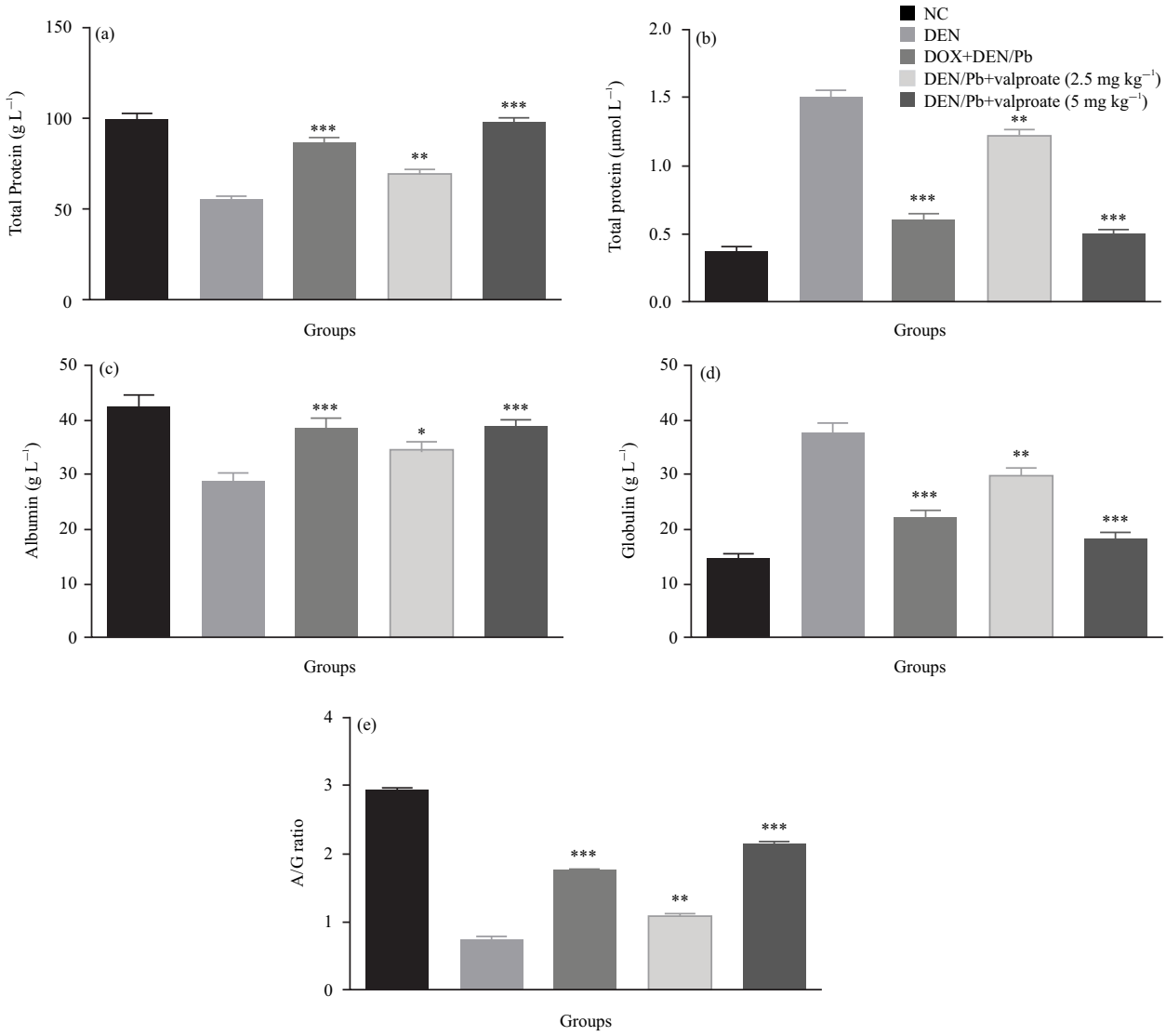

Fig. 5(a-e): Summarized the effect of valproate on the biochemical parameter of DEN/Pb induced hepatic cancer

a: Total protein, b: total bilirubin, c: Albumin, d: Globulin and e: A/G ratio. NC: Normal control, DEN: Diethylnitrosamine, PB: Phenobarbital, NS: Non-significant. Data represent the Mean±SD (n = 6).*p<0.05 is significant, **p<0.01 is more significant and ***p<0.001 is extremely significant

function after administration of DEN/PB. Although DEN/PB and Valproate treatment suggest a substantial decrease in serum ALT levels compared with that in normal control rats. In the same way, in a group treated with Valproate and DOX, the level of ALP (Fig. 4c) was observed to be decreased, whereas the reduction in the value was not considerably different compared with the control rats.

In HCC rats treated with Valproate and DOX, the level of total protein (Fig. 5a), total bilirubin (Fig. 5b), albumin (Fig. 5c), globulin (Fig. 5d) and albumin/globulin ratio (Fig. 5e) were significantly altered as compared with that in untreated HCC controls. The albumin and total protein levels were not substantially different compared with those in untreated HCC controls (Fig. 5). Besides, there was a substantial reduction in the levels of total protein, albumin and albumin/globulin ratio in the hepatic cancer rats relative to those in the normal control rats. In the hepatic cancer-treated groups, the level of total bilirubin was significantly enhanced relative to that in the control group.

Effect on serum electrolytes, creatinine and urea level: The level of serum electrolyte urea and creatinine were analyzed in rats affected with HCC after 28 days of management with both dose levels of Valproate and DOX. Results indicate that hepatic cancer-induced rats were treated with DOX and Valproate with DOX, did not cause a significant alteration in

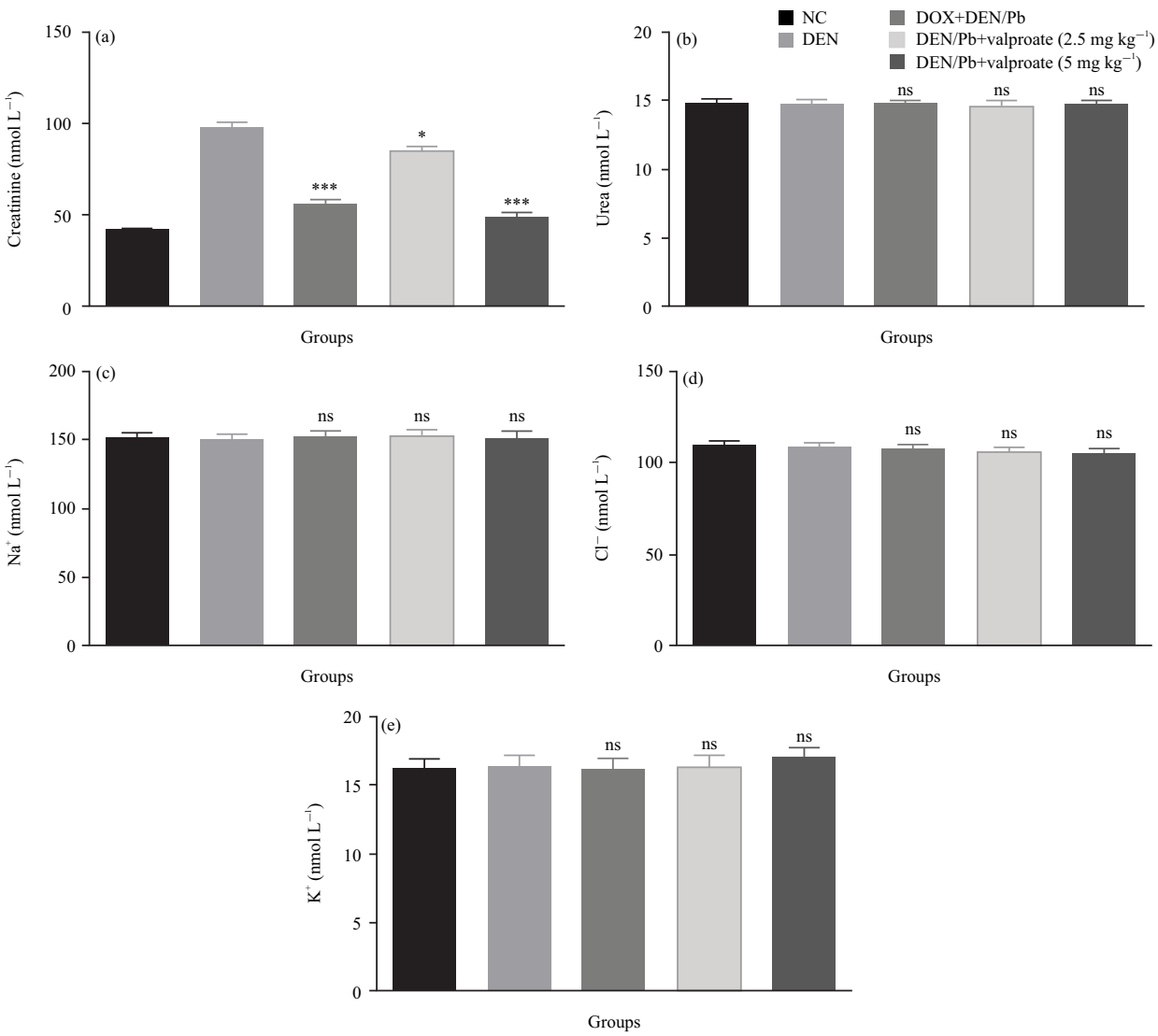

Fig. 6(a-e): Effect of valproate on the serum electrolytes, creatinine and urea of DEN/Pb induced hepatic cancer

a: Creatinine, b: Urea, c: Na^+, d: Cl^- and e: K^+. NC: Normal control, DEN: Diethylnitrosamine, PB: Phenobarbital, Na^+: Sodium, Cl^-: Chlorine, K^+: Potassium, NS: Non-significant. Data represent the Mean±SD (n = 6).*p<0.05 is significant, **p<0.01 is more significant and ***p<0.001 is extremely significant

the levels of creatinine (Fig. 6a),urea (Fig. 6b), Na^+ (Fig. 6c), Cl^- (Fig. 6d) and K^+ (Fig. 6e) levels compared to normal control rats. Whereas, K^+ level in DEN/PB induced HCC rats treated with DOX and Valproate was significantly higher than control group rats (Fig. 6).

Effect on glycoprotein levels: Our findings showed a substantial reduction in the levels of hexose (Fig. 7a), hexosamine (Fig. 7b), fucose (Fig. 7c) and sialic acid (Fig. 7d) in the tissue following treatment with DEN/Pb, whereas administration valproate at both dose levels revealed a significant degree of restoration of all four glycoproteins relative to the DEN/Pb induced group. Administration of standard drug yielded similar results compared with the control group (Fig. 7).

Oxidative stress: In comparison to control, DEN/Pb treatment significantly increased the level of lipid peroxidation content in the liver tissue (Fig. 8a). Supplementation of Valproate in DEN/Pb induced groups outfall into a significant reduction in lipid peroxidation levels and the values of PCC compared to rats treated with DEN/Pb alone. Valproate therapy showed no major harm as opposed to control on membrane lipids and proteins. In DEN/Pb-treated rats, the activities of GSH (Fig. 8b),

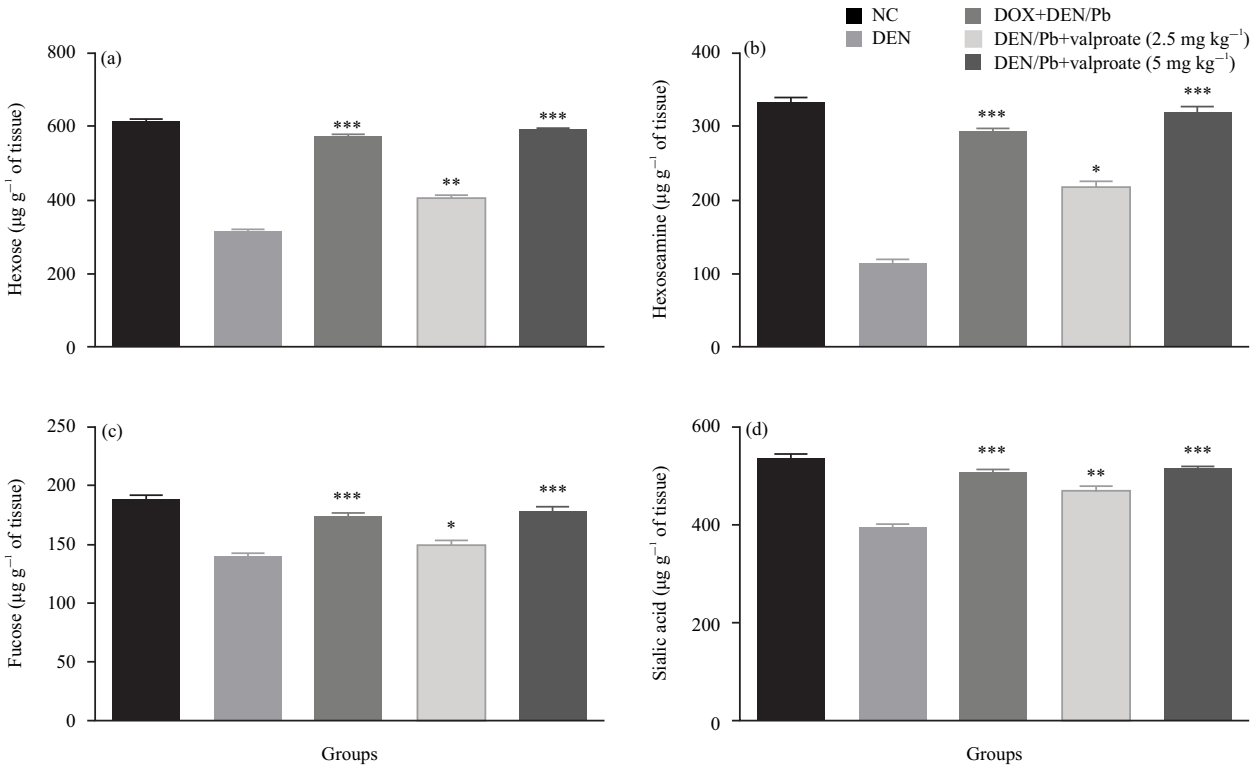

Fig. 7(a-d): Summarized the effect of valproate on the glycoprotein level of DEN/Pb induced hepatic cancer

a: Hexose, b: Hexosamine, c: Fucose and d: Sialic acid. NC: Normal control, DEN: Diethylnitrosamine, PB: Phenobarbital, NS: Non-significant. Data represent the Mean±SD (n = 6). *p<0.05 is significant, **p<0.01 is more significant and ***p<0.001 is extremely significant

SOD (Fig. 8c), CAT (Fig. 8d), GST (Fig. 8e) and Gpx (Fig. 8f) were considerably reduced in contrast with control rats. In contrast, large changes in the expression of these enzymes were observed in animals co-treated with Valproate and DEN/Pb relative to the group treated with DEN/Pb induced hepatic cancer rats.

Cytokines and inflammatory mediators: DEN/Pb induced rats showed an increased level of cytokines such as TNF-α (Fig. 9a), IL-6 (Fig. 9b) and IL-1β (Fig. 9c) and inflammatory mediator include NF-κB (Fig. 9d). Valproate significantly reduced the cytokines and inflammatory mediators (Fig. 9).

TGF-β1: To investigate the mechanisms underlying Valproate's preventive effect on hepatic cancer, we planned Valproate could defend the liver from cancer caused by DEN/Pb by repressing the liver inflammation. ELISA has examined the levels of TGF-β1 (cytokine profibrogenic agent) in the liver. In rats treated with DEN/Pb showed a significant enhancement was observed in the TGF-β1 as depicted in Fig. 10. Administration of Valproate substantially reduced the TGF-β1 levels in comparison with hepatic cancer rats. The standard drug DOX also significantly the levels of TNF-α and TGF-β1 levels.

DISCUSSION

The findings of biomarkers of tumours, liver characteristics and body weight are the main basic indicators in the initial assessment of the pathology of the liver. HCC rats induced by DEN and pB used as promoter reveal loss of body weight in this project, which confirmed previous findings. They said that the model caused by cancer showed a reduced weight gain in comparison to the normal control rats. The rats showed benign hepatomas after cancer induction with DEN/PB, which is similar to neoplastic nodules and recognized as a potent precursor for the development of HCC in the experimental models of animals.

The liver collected from HCC induced rats indicated substantial up-regulation in body weight in the normal group relative to that and may impact PB liver rats[14]. PB administration is interconnected with rodents initial transient

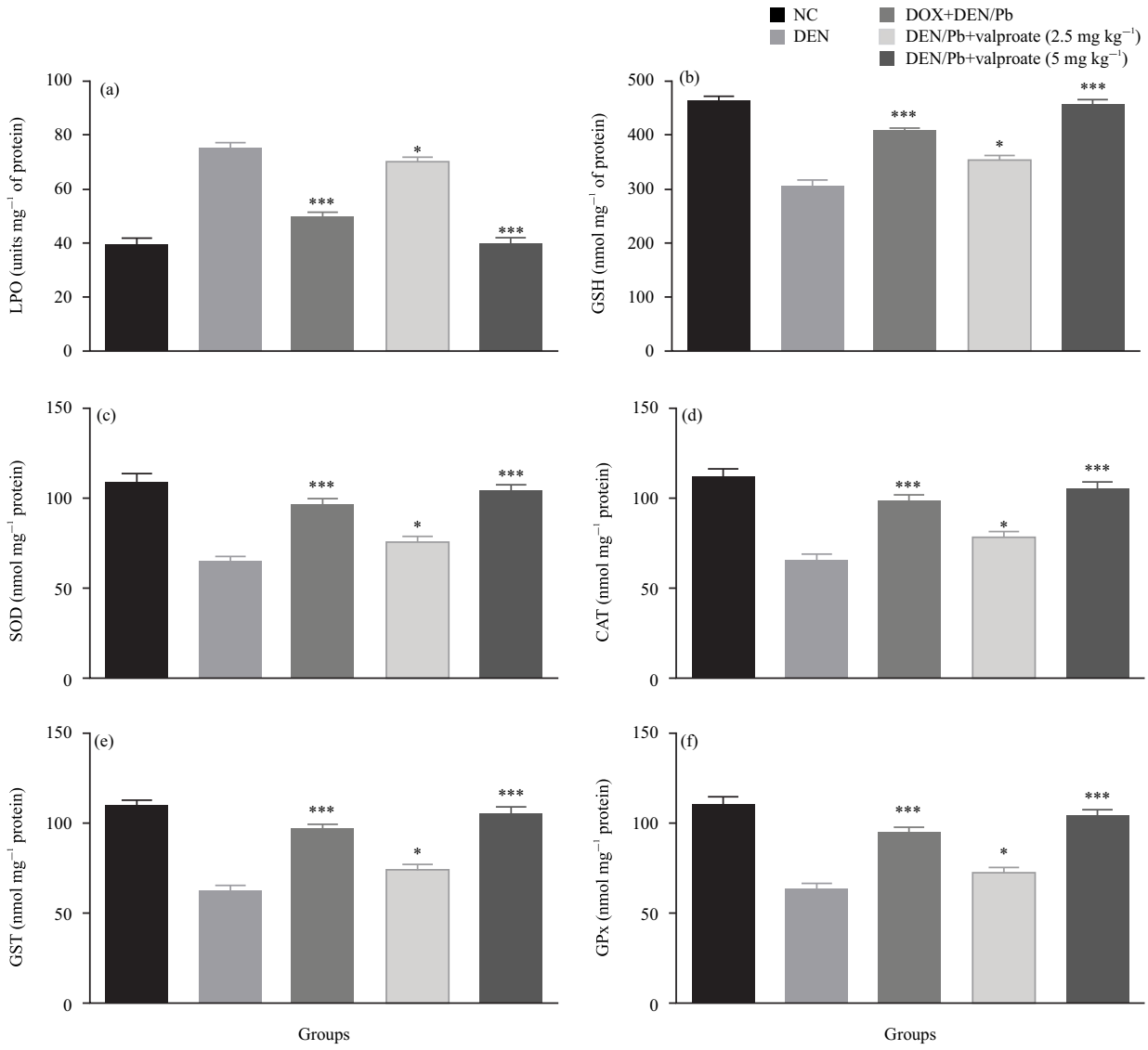

Fig. 8(a-f): Effect of valproate on the antioxidant parameters of DEN/Pb induced hepatic cancer

a: LPO, b: GSH, c: SOD, d: CAT, e: GST and f: GPx. NC: Normal control, DEN: Diethylnitrosamine, PB: Phenobarbital, LPO: Lipid peroxidation, GSH: Glutathione, SOD: Superoxide dismutase, CAT: Catalase, GST: Glutathione-s-transferase, Gpx: Glutathione peroxidase, NS: Non-significant. Data represent the Mean±SD (n = 6).*p<0.05 is significant, **p<0.01 is more significant and ***p<0.001 is extremely significant

hyperplasia before time post-treatment case and smooth endoplasmic reticulum proliferation leads to hepatocellular hypertrophy, which adds to the increase in liver weight[14]. Administration of Valproate, however, decreases the weight of the liver, which gives a hint about hepatic improvement. This finding was consistent with that in earlier research[10,14].

Alpha-fetoprotein is a valuable diagnostic tumour producer of HCC used for tumour staging, treatment, tracking and even detection of liver tumour recurrence[3,7,15]. In this study, a significant increase in the αFP level was observed during the induction of HCC using DEN and 20 weeks of PB administration[16]. The αFP level was significantly enhanced in DEN induced HCC rats. Nonetheless, after 28 days of treatment with Valproate, the amount of αFP substantially decreases, indicating Valproate's anticancer activity against HCC in rats. Besides, major increases in AST, ALT and ALP behaviours were observed in DEN/PB-induced rats. DEN/PB-induced liver cancer deteriorates the membrane of a cell, directs to the transaminases and phosphatase leakage (as the main location in the liver) from the tissue of the liver into the stream of blood[17,18]. Hepatic cancer rats treated with Valproate the d DOX suggests remarkably lowering of AST, ALP and ALT (liver

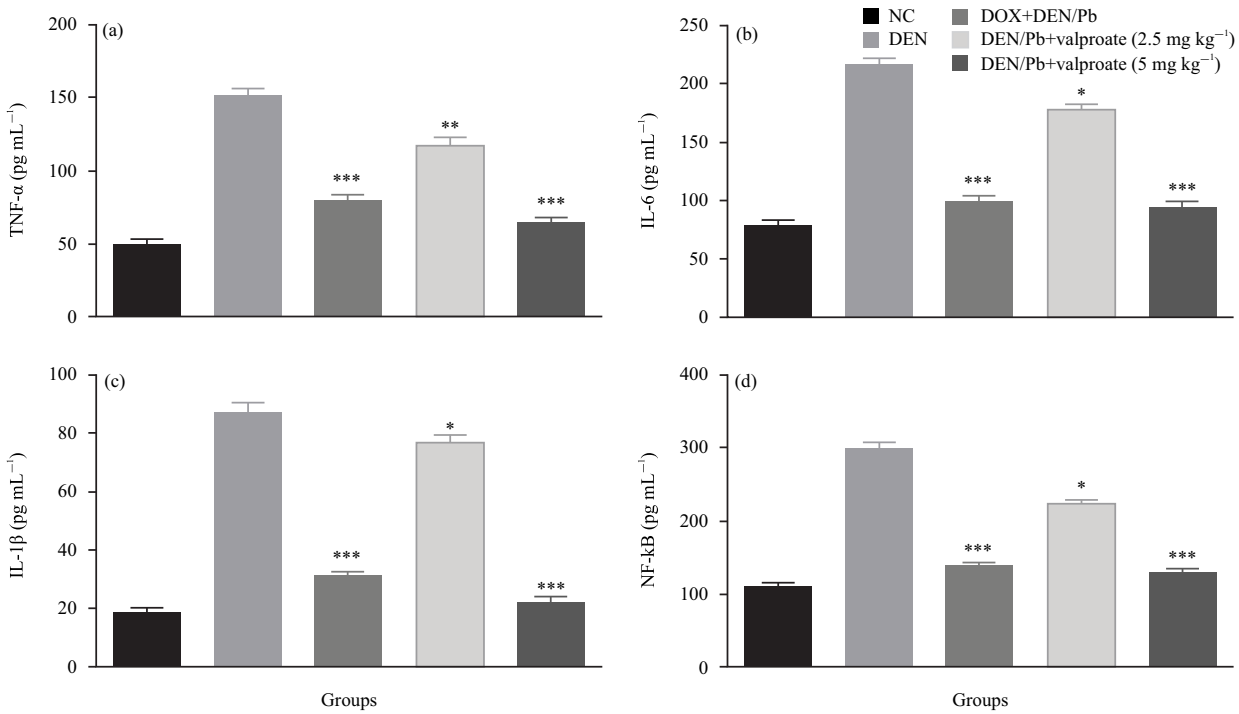

Fig. 9(a-d): Effect of valproate on the cytokines and inflammatory parameters of DEN/Pb induced hepatic cancer

a: TNF-α, b: IL-6, c: IL-1β and d: NF-κB. NC: Normal control, DEN: Diethylnitrosamine, PB: Phenobarbital, TNF-α: Tumor necrosis factor-α, IL-6: Interleukin-6, IL-1β: Interleukin-1β, NF-κB: Nuclear kappa B factor, NS: Non-significant. Data represent the Mean±SD (n = 6).*p<0.05 is significant, **p<0.01 is more significant and ***p<0.001 is extremely significant

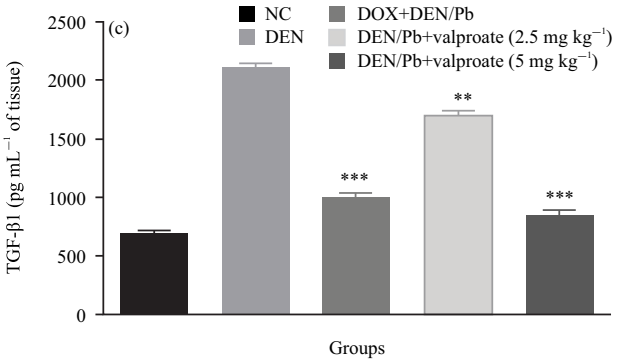

Fig. 10: Effect of valproate on the TGF-β1 level of DEN/Pb induced hepatic cancer

NC: Normal control, DEN: Diethylnitrosamine, PB: Phenobarbital, NS: Non-significant. Data represent the Mean±SD (n = 6).**p<0.01 is more significant and ***p<0.001 is extremely significant

enzymes) relative to the control rats. This signifies Valproate ability to improve the injury of liver injury by retaining the cell membrane integrity, thus alleviating carcinogenic progression.

Serum bilirubin level is recognized as an indicative marker of liver function in the monitoring of health and ailment[13]. Treatment with DEN/PB caused damage to the liver of rat out falls in the rise in the amount of conjugated bilirubin, suggesting a pathological shift in the flow of biliary fluid[13]. Although, Valproate therapy enhances the effect by reducing the overall level of bilirubin, indicating the pharmacological potential of the Valproate in rats suffers from HCC.

The biochemical characteristics of the synthetic ability of the liver as diagnostic criteria are two serum protein levels globulin and albumin[14]. Treatment with DEN/PB has altered the Albumin/Globulin (A/G) ratio, an indicator of extreme deficiency in the liver's protein biosynthesis[19]. The variability of the total serum protein content found in this research confirms the DEN and PB mutagenic action[20]. DEN causes level polyribosomes to dissociate from the endoplasmic reticulum surface that plays a crucial role in the biosynthesis of proteins.

This alteration interferes with liver protein synthesis that is close to the earlier research[16] after DEN/PB administration. The A/G ratio was reversed to a slightly better level in rats being treated with Valproate at both dose levels because of the increased serum globulin level relative to that in the DEN induced HCC. People who have already had hepatic cancer and other venerable liver damage experience hepato-renal syndrome in patients with liver disease suffering kidney dysfunction[21]. Therefore, the measurement of content urea, creatinine and electrolyte in rats were examined with

Valproate and standard drug DOX. The level of electrolyte and urea remained almost the same compared to control group rats, except potassium (K^+) level, which appeared to be high in the DEN/PB-induced HCC rats. A higher serum K^+ level suggests intracellular potassium leakage from the kidney tubular epithelium into the stream of blood or a decrease in the removal of K^+ by renal excretion.

Lipid peroxidation is the principal materialization of destruction to oxidative factors and the chief component in the DEN/PB toxicity[22]. The results from this study show that lipid peroxidation is allied with toxicity to DEN/PB and that co-treatment with Valproate has kept the range of lipid peroxidation to be minimal[23-25]. Co-treatment with Valproate decreased the levels of LPO in the liver tissue compared to DEN/PB-treated rats alone. Cells have endogenous defence mechanisms that help defend against cell damage caused by free radicals. Endogenous defence mechanisms embrace the enzymes of antioxidants. SOD and CAT work in biological systems against dangerous oxygen free radicals including superoxide (O_2) and hydroxyl ions (OH). CAT avoids oxidative exposure caused by H_2O_2 by catalyzing the H_2O and O_2 formation[3,7].

Glutathione is by itself a non-enzyme antioxidant and straightly scavenges reactive oxygen species, for example, lipid peroxides[26]. In the existence of GSH, GPx and GST effectively quench the toxin-induced free radicals and heavy metals[8,9]. Accordingly, in this study, the estimation of the above enzymatic and non-enzymatic antioxidant activities confirms Valproate's protective effect on DEN/PB-induced oxidative stress.

Glycoproteins are naturally conjugated proteins where carbohydrate molecules are covalently coupled with polypeptide asparagine or serine or threonine residues[27]. Glucose, fucose, galactose, sialic acid, mannose and a few acetylated derivatives of hexosamine are the main sugar molecules present in glycoproteins. Glycoconjugates are an essential component of the cell membrane and play an important role in cell and cell membrane functioning[28]. Increased glycoconjugates in circulation were recorded by increased turning, secretion and detaching from damaged cells or malignant.

The decrease in hexose, fucose, hexosamine and sialic acid levels in the tissue of liver tissue following treatment with DEN/PB suggests that administration of carcinogenic agents affects these conjugated proteins glycoproteins. In rodents, co-administration of Valproate and DEN/PB, glycoprotein levels in liver tissue were significantly enlarged as opposed to rats treated with DEN/PB alone. The outcomes of this study advocate that Valproate, with its cytoprotective action, prevents damage to DEN induced liver tissue, thus restraining the liver tissue glycoprotein levels to almost close to the value of the control group.

TNF-α is the main component triggering an inflammatory cascade including cytokine induction following hepatic injury[5,29]. The damaged liver, primarily Kupffer cells, neutrophils and macrophages that infiltrate, will develop TNF-α[4,13]. In the liver, TNF-α plays a dichotomous role not only in the apoptosis, inflammation and proliferation of hepatocytes but also in the repression of the α1 gene expression in collagen[30,31]. Our findings showed that oral administration of treatment with Valproate and DOX decreased the TNF-α level in hepatic tissue as contrast with DEN/PB given group alone. This finding indicated that the defensive property of Valproate on DEN/PB induced HCC was at least in part attributable to their ability to suppress TNF-α development. TGF-β1, released from Kupffer cells and oxidative stress induced by DEN/PB activated HSC activation[20]. HSC activation can also be caused by numerous chronic injuries to the liver, such as toxins, viral hepatitis and autoimmune disorders[32,33]. TGF-β1 is a major cytokine with profibrogenic. Its principal functions are the control of ECM growth, degradation and accumulation in liver fibrosis. TGF-β1 contributes to fibrogenesis via HSC's autocrine and paracrine effects[34]. Our analysis suggests that treatment with Valproate and DOX decreased the TGF-β1 levels in the tissue of the liver as comparison with DEN/PB induced the HCC group. This indicated that the defensive nature of Valproate on DEN/PB induced HCC was related to their ability to stop the activation of HSC by minimizing TGF-β1 output.

CONCLUSION

Based on the result, we can say that Valproate exhibited the chemoprotective effect against HUH-7 cell lines. Valproate considerably suppressed the hepatic nodules and hepatic tissue weight. Valproate considerably decreased the α-fetoprotein and hepatic marker in DEN induced hepatic cancer. Valproate significantly reduced the LPO level and increased the level of SOD, GPx, GST, CAT, SOD. Valproate significantly decreased the cytokines and inflammatory mediators. Valproate also reduced the level of TGF-β1. Based on the result, we can say that Valproate is the best choice to treat hepatic cancer.

SIGNIFICANCE STATEMENT

This study discovers the possible hepatoprotective effect of valproate that can be beneficial for hepatic cancer and this study will help the researchers to uncover the critical areas of hepatocellular carcinoma that many researchers were not able to explore. Thus, a new theory on hepatocellular carcinoma may be arrived at in this experimental study.

REFERENCES

1. Krishnan, P., J. Sundaram, S. Salam, N. Subramaniam, A. Mari, G. Balaraman and D. Thiruvengadam, 2020. Citral inhibits N-nitrosodiethylamine induced hepatocellular carcinoma via modulation of antioxidants and xenobiotic metabolizing enzymes. Environ. Toxicol., 35: 971-981.
2. Afzal, M., I. Kazmi, R. Khan, P. Rana and V. Kumar *et al*., 2017. Thiamine potentiates chemoprotective effects of ibuprofen in DEN induced hepatic cancer via alteration of oxidative stress and inflammatory mechanism. Arch. Biochem. Biophys., 623: 58-63.
3. Pandey, P., M. Rahman, P.C. Bhatt, S. Beg and B. Paul *et al*., 2018. Implication of nano-antioxidant therapy for treatment of hepatocellular carcinoma using PLGA nanoparticles of rutin. Nanomed., 13: 849-870.
4. Kumar, V., P.C. Bhatt, M. Rahman, G. Kaithwas and H. Choudhry *et al*., 2017. Fabrication, optimization and characterization of umbelliferone β-D-galactopyranoside-loaded PLGA nanoparticles in treatment of hepatocellular carcinoma: *In vitro* and *in vivo* studies. Int. J. Nanomed., 12: 6747-6758.
5. Kumar, V., P.C. Bhatt, M. Rahman, F.A. Al-Abbasi, F. Anwar and A. Verma, 2017. Umbelliferon-α-d-glucopyranosyl-(2I→1II)-α-Dglucopyranoside ameliorates Diethylnitrosamine induced precancerous lesion development in liver via regulation of inflammation, hyperproliferation and antioxidant at pre-clinical stage. Biomed. Pharmacother., 94: 834-842.
6. Verma, A., B. Ahmed, F. Anwar, M. Rahman and D.K. Patel *et al*., 2017. Novel glycoside from *Wedelia calendulacea* inhibits diethyl nitrosamine-induced renal cancer via downregulating the COX-2 and PEG_2 through nuclear factor-κB pathway. Inflammopharmacology, 25: 159-175.
7. Rahman, M., S.A. Al-Ghamdi, K.S. Alharbi, S. Beg and K. Sharma *et al*., 2019. Ganoderic acid loaded nano-lipidic carriers improvise treatment of hepatocellular carcinoma. Drug Delivery, 26: 782-793.
8. Jayakumar, S., A. Madankumar, S. Asokkumar, S. Raghunandhakumar, S. Kamaraj, M.G.J. Divya and T. Devaki, 2012. Potential preventive effect of carvacrol against diethylnitrosamine-induced hepatocellular carcinoma in rats. Mol. Cell. Biochem., 360: 51-60.
9. Shaban, N.Z., M.A.L. El-Kersh, F.H. El-Rashidy and N.H. Habashy, 2013. Protective role of punica granatum (pomegranate) peel and seed oil extracts on diethylnitrosamine and phenobarbital-induced hepatic injury in male rats. Food Chem., Food Chem., 141: 1587-1596.
10. Chang, W., W. He, P.P. Li, S.S. Song, P.F. Yuan, J.T. Lu and W. Wei, 2016. Protective effects of *Celastrol* on diethylnitrosamine-induced hepatocellular carcinoma in rats and its mechanisms. Eur. J. Pharmacol., 784: 173-180.
11. Su, X.Y., J.Q. Zhao, N. Li, M. Kumar and A.M.O. Yang, 2019. Chemoprotective effects of resveratrol against diethylnitrosamine induced hepatocellular carcinoma in wistar rats. Int. J. Pharmacol., 15: 549-559.
12. Song, J., W. Ding, B. Liu, D. Liu and Z. Xia *et al*., 2020. Anticancer effect of caudatin in diethylnitrosamine-induced hepatocarcinogenesis in rats. Mol. Med. Rep., 22: 697-706.
13. Rahman, M., W.H. Almalki, O. Afzal, I. Kazmi and A.S.A. Altamimi *et al*., 2021. Diosmin-loaded solid nanoparticles as nano-antioxidant therapy for management of hepatocellular carcinoma: QbD-based optimization, *in vitro* and *in vivo* evaluation. J. Drug Delivery Sci. Technol., Vol. 61. 10.1016/j.jddst.2020.102213.
14. Mohamed, N.Z., H.F. Aly, H.A. moneim El-Mezayen and H.E. El-Salamony, 2019. Effect of co-administration of bee honey and some chemotherapeutic drugs on dissemination of hepatocellular carcinoma in rats. Toxicol. Rep., 6: 875-888.
15. Anwar, F., R. Khan, R. Sachan, I. Kazmi and A. Rawat *et al*., 2019. Therapeutic role of calcium and vitamin K3 in chemically induced hepatocarcinogenesis-new tools for cancer treatment. Arch. Physiol. Biochem., 125: 270-275.
16. Abozaid, O.A.R., F.S.M. Moawed, M.A. Farrag and R.S.M. Kawara, 2020. Synergistic effect of benzethonium chloride combined with endoxan against hepatocellular carcinoma in rats through targeting apoptosis signaling pathway. Asian Pac. J. Cancer Prev., 21: 1709-1716.
17. Karabekir, S.C. and A. Özgörgülü, 2020. Possible protective effects of resveratrol in hepatocellular carcinoma. Iran J. Basic Med. Sci., 23: 71-78.
18. Adikwu, E, N.C. Ebinyo and O. Benalayefa, 2020. Protective effect of lycopene against tamoxifen-induced hepatotoxicity in albino rats. Biomed. Biotechnol. Res. J., 4: 69-75.
19. Punvittayagul, C., A. Chariyakornkul, K. Jarukamjorn and R. Wongpoomchai, 2021. Protective role of vanillic acid against diethylnitrosamine- and 1,2-dimethylhydrazine-induced hepatocarcinogenesis in rats. Molecules, Vol. 26. 10.3390/molecules26092718.
20. Alansari, W.S. and A.A. Eskandrani, 2020. The anticarcinogenic effect of the apple polyphenol phloretin in an experimental rat model of hepatocellular carcinoma. Arabian J. Sci. Eng., 45: 4589-4597.
21. Özeren, N., M.A. Kisacam, G.O. Kocamuftuoglu, N. Kaya and S.T. Ozan, 2019. The protective role of oleuropein against diethylnitrosamine and phenobarbital induced damage in rats. Turk. J. Biochem., 44: 714-721.

22. Momen, Y.S., R.M. Hussein and M.A. Kandeil, 2019. Involvement of PI3K/Akt pathway in the protective effect of hesperidin against a chemically induced liver cancer in rats. J. Biochem. Mol. Toxicol., Vol. 33. 10.1002/jbt.22305.
23. You, Y., F. Zhu, Z. Li, L.F. Zhang and Y. Xie *et al*., 2021. Phyllanthin prevents diethylnitrosamine (DEN) induced liver carcinogenesis in rats and induces apoptotic cell death in HepG2 cells. Biomed. Pharmacother., Vol. 137. 10.1016/j.biopha.2021.111335.
24. Sun, Z., L. Sun and W. Li, 2020. Effect of ganoderic acid on diethylnitrosamine-induced liver cancer in mice. Trop. J. Pharm. Res., 19: 2639-2644.
25. Dar, K.K., S. Ali, M. Ejaz, S. Nasreen and N. Ashraf, 2019. *In vivo* induction of hepatocellular carcinoma by diethylnitrosoamine and pharmacological intervention in Balb C mice using *Bergenia ciliata* extracts. Braz. J. Biol., 79: 629-638.
26. Singh, D., E. Yadav, N. Falls, V. Kumar, M. Singh and A. Verma, 2019. Phytofabricated silver nanoparticles of phyllanthus emblica attenuated diethylnitrosamine-induced hepatic cancer via knock-down oxidative stress and inflammation. Inflammopharmacology, 27: 1037-1054.
27. Elguindy N.M., G.A. Yacout, E.F. El Azab and H.K. Maghraby, 2016. Chemoprotective effect of *elettaria cardamomum* against chemically induced hepatocellular carcinoma in rats by inhibiting NF-κB, oxidative stress and activity of ornithine decarboxylase. S. Afr. J. Bot., 105: 251-258.
28. McLoughlin, M.R., D.J. Orlicky, J.R. Prigge, P. Krishna and E.A. Talago *et al*., 2019. TrxR1, Gsr and oxidative stress determine hepatocellular carcinoma malignancy. Proc. Nat. Acad. Sci., 116: 11408-11417.
29. Rahman, M., W.H. Almalki, O. Afzal, A.S.A. Altamimi and I. Kazmi *et al*., 2020. Cationic solid lipid nanoparticles of resveratrol for hepatocellular carcinoma treatment: Systematic optimization, *in vitro* characterization and preclinical investigation. Int. J. Nanomed., 15: 9283-9299.
30. Ding, Y.F., Z.X. Peng, L. Ding and Y.R. Peng, 2019. Baishouwu extract suppresses the development of hepatocellular carcinoma via TLR4/MyD88/NF-κB pathway. Front. Pharmacol., Vol. 10. 10.3389/fphar.2019.00389.
31. Usman, M.S., M.Z. Hussein, A.U. Kura, S. Fakurazi, M.J. Masarudin and F.F.A. Saad, 2018. Synthesis and characterization of protocatechuic acid-loaded gadolinium-layered double hydroxide and gold nanocomposite for theranostic application. Appl. Nanosci., 8: 973-986.
32. Fikry, R., N. Zein and A. Faozan, 2018. Properties of crocus sativus saffron on DEN-induced hepatocellular carcinoma in rats. Biochem. Lett., 13: 171-179.
33. Li, S., H. Li, X. Xu, P.E. Saw and L. Zhang, 2020. Nanocarrier-mediated antioxidant delivery for liver diseases. Theranostics, 10: 1262-1280.
34. Mondal, M., M.M. Hossain, M.R. Hasan, M.T.I. Tarun and M.A.F. Islam *et al*., 2020. Hepatoprotective and antioxidant capacity of *Mallotus repandus* ethyl acetate stem extract against d-Galactosamine-induced hepatotoxicity in rats. ACS Omega, 5: 6523-6531.