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ORIGINAL ARTICLE

Anticancer Potential of Vernodalin on Diethylnitrosamine Induces Liver Cancer by Inhibition of Cell Proliferation

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ABSTRACT

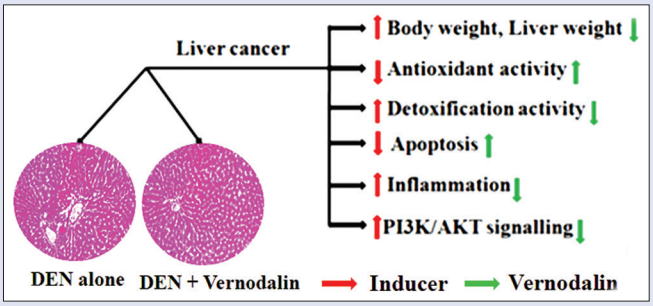
Background: Hepatocellular carcinoma (HCC) is the utmost familiar form of liver distortion, a prevalent high incidence that ensures the fifth most recurrent primary malignancy around the world. **Objectives:** To investigate the chemopreventive and apoptotic effect of vernodalin in diethylnitrosamine (DEN) induced HCC. **Materials and Methods:** Rats were distributed into four sets of experimental animals every six rats: control group, DEN alone, DEN + vernodalin, (10 mg/kg bw), and vernodalin (10 mg/kg bw) alone. The analysis was conducted to measure the liver and body weight. The assay was designed to analyze hepatic toxic markers, antioxidants, lipid peroxides, and inflammatory cytokines, in addition, histopathology and western blot analysis were also conducted. **Results:** DEN with vernodalin diminished weight of the body and antioxidant level whereas raised the liver weight, toxic marker concentration, oxidative stress and cytokines. Vernodalin also prevents oxidative stress, lipid peroxides level, toxic markers, pro-inflammatory, and cytokines. Vernodalin suppressed the P13K/AKT signaling that enhances apoptosis and maintains the morphology of cells. **Conclusion:** The results indicate that vernodalin exerts a promising chemopreventive and apoptotic natural agent for DEN-induced HCC in rats.

Key words: Chemoprevention, diethylnitrosamine, hepatocellular carcinoma, P13K/AKT signalling, Vernodalin

SUMMARY

- Vernodalin treatment maintains body weight, liver weight, and antioxidant levels of DEN induced rats
- Vernodalin suppressed the PI3K/AKT signaling pathways
- Vernodalin induced apoptosis in DEN induced rats.

Abbreviations used: DEN: Diethylnitrosamine; HCC: Hepatocellular carcinoma; ROS: Reactive oxygen species; H&E: Hematoxylin and eosin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase; γGT: Gamma glutamyltranspeptidase; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; GR: Glutathione reductase; GST: Glutathione-S-transferase.



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INTRODUCTION

Hepatocellular carcinoma (HCC) is accountable for the third malignant-allied mortality worldwide, estimated that, by 2025, >1 million individuals will be affected.[1,2] Numerous risk factors include: chronic HBV infection, chronic HCV infection, NAFLD and NASH, alcoholic liver disease, hereditary hemochromatosis, and any other causes leading to cirrhosis. The principal risk factors associated with HCC are alcohol consumption, aflatoxin B1, hormone exposure, hemochromatosis, Hepatitis B and C, pollutants, and diethylnitrosamine (DEN).[3] The environmental cancer-initiating compound DEN is also actively contributing to the initiation of liver tumours. DEN prompts fitness problems in humans and primarily exists in whiskey, tobacco, processed meat, pharmaceuticals, and cosmetics.[4,5] DEN triggers progressive, proliferative, neoplastic lesions and an extremely mutagenic hepatic tumour.

DEN is comprised of carcinogenic compounds N-nitrosamine present in various foods,[6] and its carcinogenicity is renowned for the DNA alkylating effect.[7] Hepatic carcinogens create free radicals responsible for oxidative stress and interfere with redox balance, which stimulates antioxidative repair mechanisms.[8] HCC activates the cytochromes P450 (CYP450) enzymes to generate the reactive oxygen species (ROS) in the liver.[9] The prolonged exposure to DEN promotes hepatic necrosis and ultimately leads to liver failure. Notably, the free radical assembly has been activated by the PI3K/Akt cell-survival signaling cascades.[10,11] As a result, HCC induced by DEN mimics the human HCC with equivalent

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sequential progression. Moreover, the molecular mechanisms elaborate in liver cancer are well recognized in DEN-induced HCC rats.[12]

Comprehensive research was focused to discover the unique bimolecular from conventional sources as a chemopreventive agent that restrains the various paces in cancer. Recently, natural compounds such as umbelliferone,[13] syringic acid,[14] and nerolidol,[15] have substantial prominence, due to their huge application as an antioxidant and to prevent the oxidative devastation of cells. Vernodalin is a sesquiterpene lactone isolated from numerous medicinal plants. It exerts cytotoxic, apoptotic, antioxidant, and anticancer activity against various cancers.[16,17] Pratheesh Kumar *et al.*, 2011[18], has been demonstrated that vernolide induces apoptosis by regulation of pro-inflammatory cytokines in B16F-10 melanoma cells. Nguyen *et al.*, 2020,[19] reported vernolide has potent cell proliferation, metastasis, angiogenesis, and apoptosis property in cancer. Looi *et al.*, 2013,[20] demonstrated that vernodalin treatment with 10 mg/kg/bw suppressed the development of breast tumors via down-regulation of PI3K/Akt signalling pathways. From this study, 10 mg/kg bw were selected for our dose fixation study for current research. However, the molecular action of vernodalin (10 mg/kg/bw) on DEN-induced HCC has not been fully elucidated. Therefore, we explored the chemopreventive effect on cell proliferation and apoptotic pathway of vernodalin through PI3K/Akt signalling in the DEN-prompted HCC model.

MATERIALS AND METHODS

Chemicals

Diethylnitrosamine, Vernodalin, Phosphate buffer saline, EDTA, and Tris buffer were bought from Merck, Germany. ELISA assay kits were achieved from Cayman chemicals, USA. The primary antibodies (P13K, AKT, and β-actin), and secondary antibodies, were procured from Himedia Pvt Ltd, USA.

Experimental Animals

The study was carried out on Wistar albino male rats each group contains six rats, and their body weight ranged between 130 and 150 g was well-preserved in precise temperature under standard 25°C, humidity for 12 h day/night cycles. The control group animals were fed with standard rat chow and water *ad libitum*. The food was withdrawn 18–24 h before the experiment. The care and handling of laboratory animals were done according to the guidelines of the Shaanxi Provincial Hospital of Traditional Chinese Medicine animal ethical committee (Approved No. 202100) under Good Laboratory Practice (GLP).

Induction of HCC

DEN (0.01%) is used for inducing HCC in rats described by the method reported previously by Sivaramakrishnan *et al.*, 2007.[21]

Experimental design

The Wistar albino rats were separated into four sets of six each. Group I control rats, in group II was given DEN (0.01%) in drinking water. Group III was provided DEN (0.01%) + vernodalin (10 mg/kg bw) and group IV was administered with vernodalin (10 mg/kg bw) alone. Bodyweight was recorded during the trial. Finally, the rats were preserved by fasting overnight and then euthanized by decapitation. The blood and liver samples were collected in ice-cold dishes for biochemical analysis.

Tissue homogenate preparation

In the experimental animal section (The food was withdrawn 18–24 h before the experiment for the control group). The liver was detached, cleaned with saline, weighed, and evened out with 0.9% NaCl, centrifuged at 4°C for 20 min. The supernatant was collected for biochemical analysis.

Histopathological assessment of exploration of liver

The hepatic tissues were static with 10% formaldehyde and subsequently implanted in the paraffin wax. By using a rotary microtome, cut the liver tissues into 3–5 μm sections and successively stain with H & E stain. Hepatocytes alterations were perceived through a light microscope. The slides stained with hematoxylin-eosin were photographed using a loupe in the image and necrosis in the entire tumour was analyzed using the WinROOF software package.

Assay of cancer markers enzymes in the liver

The hepatic enzymes marker alkaline phosphatase (ALP), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transpeptidase (γGT) were used to conduct the assay according to the instructions of Randox (Randox Laboratories Ltd., Antrim, UK). The absorbance was recorded using the PerkinElmer spectrophotometer, Lambda 25 UV/VIS.

Antioxidant enzymes assays

The antioxidant enzyme status of hepatic SOD,[22] CAT,[23] GPx,[24] GR,[25] and GST,[26] were determined using the method of spectrophotometry. The absorbance was recorded during the PerkinElmer spectrophotometer, Lambda 25 UV/VIS.

Lipid peroxidation (LPO) measurements

TBARS were assessed according to the technique of Ohkawa, 1979,[27] Lipid hydroperoxides,[28] conjugated dienes (CD).[29] The absorbance has been reported with the PerkinElmer spectrophotometer, Lambda 25 UV/VIS for all measurements.

Determination of cytokines in serum

The cytokines namely TNF-α, IL-6, IL-1β, and NF-κB in serum were measured by the ELISA kits obtained from Cayman Chemicals, USA. The concentration was expressed as pg/mL.

Western blot analysis

The P13K/AKT expression was analyzed by western blot. Briefly, the rat liver was crushed with lysis buffer and centrifuged at 4°C at 10,000 g. Pooled the supernatant and whole protein were assessed by the Bradford technique.[30] The protein (20 μg) was electrophoresed by SDS-PAGE and shifted to nitrocellule membrane. About 3% bovine serum albumin was added to block the membrane for 2 h. Added primary antibodies followed by the secondary antibody for 60 min, and blots were detected with ImmunoStar LD (Wako Pure Chemical Ind., Tokyo, Japan). Immunoreactive bands were visualized by using FluorChem™ FC3 (Protein Simple, San Jose, CA, USA).

Statistical analysis

Data were presented as means standard deviations (SD) of three independent experiments. Statistical significance was determined using the Student's *t*-test or analysis of variance (ANOVA). Statistical significance was observed as $P \geq 0.05$.

RESULTS

Influence of vernodalin on body weight and liver weight

The diet and water intake in all groups do not show any noticeable variations. The weight of the body, and liver was lessened pointedly ($P < 0.05$) in DEN administered group against the normal. The body weight and liver weight were markedly ($P < 0.05$) high in the DEN alone group. Administration of vernodalin + DEN augmented

the body weight, and liver weight of the liver was decreased. Similar results were detected in the control and vernodalin alone administered rats [Figure 1].

Influence of vernodalin on liver marker enzymes

The level of toxicity enzymes via ALT, ALP, AST, γGT, and LDH in serum was raised expressively ($P < 0.05$) in DEN stimulated rats versus normal. These hepatic enzyme levels were dropped in the treatment of vernodalin together with DEN. Normal marker enzyme levels were detected in vernodalin treated rats [Figure 2].

Influence of vernodalin on antioxidant enzyme activity

The antioxidant enzymes for instance CAT, SOD, GPx, and GR were pointedly ($P < 0.05$) condensed in DEN-treated HCC rats versus control. Vernodalin has reverted the enzymatic antioxidant status to near normal, thereby improving the defence mechanism. Vernodalin alone treated rats showed normal antioxidant status [Figure 3].

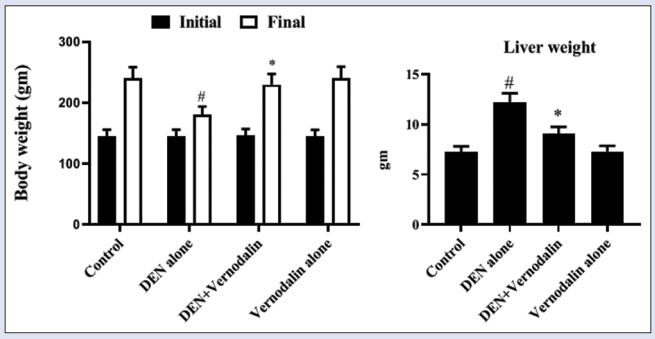

Figure 1: Effect of vernodalin on the body weights, and liver weight in control and experimental rats. Values were stated as mean ± SD of six observations. #, *$P < 0.05$ indicates the values not sharing a common superscript deliberated as significance

Impact of vernodalin on LPO

TBARS, LOOH, and CD levels were elevated remarkably ($P < 0.05$) in DEN-induced rats than in normal control. Oral supplementation of vernodalin accompanied by DEN fell considerably ($P < 0.05$) in these parameters versus DEN alone treated rats. The vernodalin alone treated rats showed similar results as the control [Figure 4].

Histopathological analysis of liver

In control, rats showed normal histology of the liver with even nuclei. HCC induced by DEN treated rats' livers described as a cytosolic region with abnormal arrangements, uneven nuclei, and fats infiltration. DEN along with vernodalin upgraded the cytosolic arrangement and the normal histology was ($P < 0.05$) prevented. Vernodalin alone treated groups exhibited normal liver histology [Figure 5].

Influence of vernodalin on cytokines

The inflammatory cytokines namely TNF-α, IL-1β, IL-6, and NF-κB were raised in DEN-driven HCC rats. These cytokine levels were significantly ($P < 0.05$) reduced by vernodalin in DEN-influenced rats. Normal rat's cytokine levels were maintained in the vernodalin alone supplementation [Figure 6].

Influence of vernodalin on P13K/Akt pathway

In the HCC development, the protein expression of P13K/Akt has a profound role in the apoptotic pathway. DEN treated rats showed P13K and Akt protein expression was significantly ($P < 0.05$) augmented in counter to control. Treatment with vernodalin in the HCC rats dropped P13K/Akt expression, whereas the vernodalin alone treated rats' protein expressions were parallel with the normal [Figure 7].

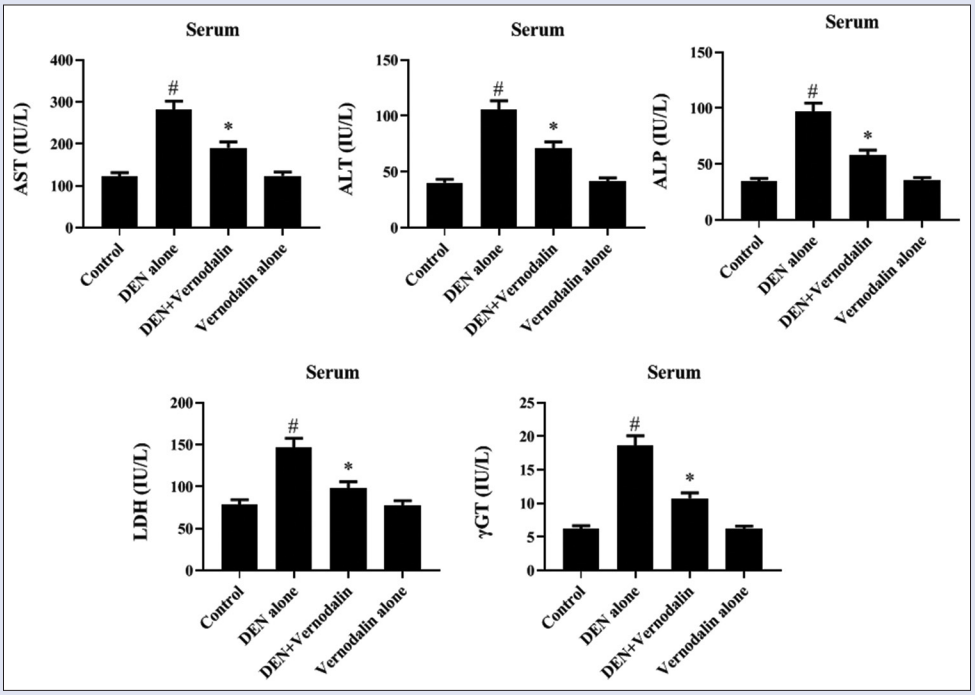

Figure 2: Effect of vernodalin on hepatic toxicity marker enzymes in serum of control and experimental rats. Results were stated as mean ± SD of six observations. **#**, * $P < 0.05$ indicates the values not sharing a common superscript deliberated as significance

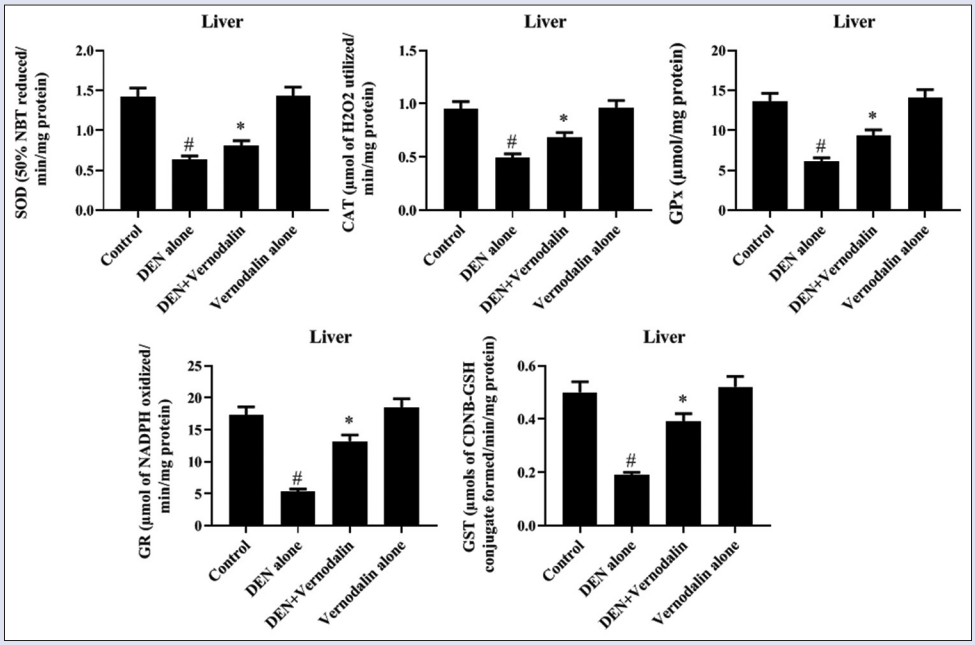

Figure 3: Effect of vernodalin on hepatic enzymatic antioxidant status on control and experimental rats. Results were stated as mean ± SD of six observations. **#**, * $P < 0.05$ indicates the values not sharing a common superscript deliberated as significance

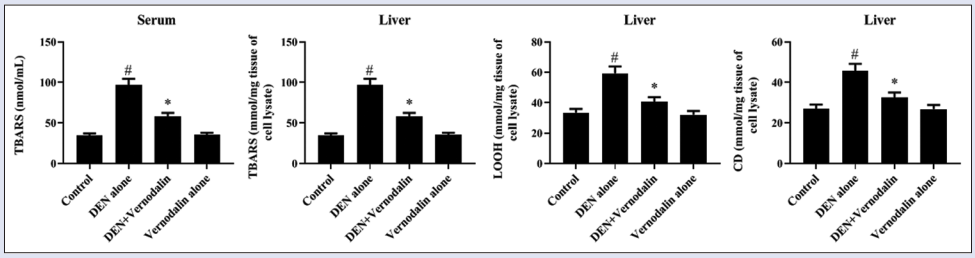

Figure 4: Effect of vernodalin on serum TBARS and hepatic TBARS, LOOH, CD in control and experimental rats. Data were stated as mean ± SD of six observations. **#**, * $P < 0.05$ indicates the values not sharing a common superscript deliberated as significance

DISCUSSION

The liver is a key vital organ, which is regulating intermediary metabolism and physiological developments in the body.[31] DEN is a typical hepatocarcinogen used to initiate liver neoplasm in a live model. DEN-initiated HCC in rats is widely used as hepatic cancer animal model.[32] The chemopreventive approach of natural products performed the possible actions including detoxification of carcinogen, subdual of mutation, inhibition of cell multiplying, apoptosis stimulation, and immune system inflexion.[33] Successfully, bioactive agents are consumed extensively in hepatic illnesses globally. Hence, we proved the shielding impact of vernodalin in DEN-induced HCC and elucidated its molecular protective actions.

Vernodalin is a well-known sesquiterpene lactone sequestered from numerous plant sources comprising *Vernonia amygdalina* and *Centratherum anthelmintica*.[34] Vernodalin demonstrates anti-bacterial, anti-malarial, and anticancer activities.[35,36] Previous studies have documented the anti-cancer and cytotoxic effect of vernodalin on ovarian cancer and melanoma cell line,[37] and nasopharynx human carcinoma.[38] This is maybe the primary report on the chemopreventive and anticancer tool of vernodalin on DEN-induced HCC.

Our findings confirmed that hepatic toxicity enzymes were elevated due to the uptake of DEN which makes ROS activity ensuing oxidative stress and cellular damage.[39] Cell destruction indicates the association of hepatic enzyme leakage into the serum. Liver impairment is analyzed by the noticeable rise in the serum activity of AST, ALT, ALP, LDH, and GGT.[40] It commences on the hepatocellular damage leads to the hepatic hypofunction and disturbance in the serum enzymes markers, with loss of membrane porousness.[41] Vernodalin administration brought back these enzymes by altering liver membrane integrity.

ROS formed due to the triggering of oxidative stress has been implicated in lipid peroxidation, DNA impairment, and mutagenesis linked with tumour formation.[42] ROS possibly will impair protein, lipid, DNA, prone to mutations, unstable chromosomes, and inflexion of cell development that may upshot to the form neoplasm. Antioxidants retain the stimulation of enzymes in drug-metabolizing that prevent hazard-persuaded mutagenesis and free radicals foraging.[43] An endogenous system of antioxidants may stabilize the ROS and diminish oxidative stress through the action of enzymatic antioxidants. In the current study, HCC animals exhibit reduced enzymatic antioxidants (SOD, CAT, GPx, GST, and GR) activities and the findings are correlated with prior findings.[44,45]

In the present findings, there was a substantial escalation of hepatic TBARS, LOOH, and CD in DEN treated rats, which confirms the inception of oxidative stress in the liver. Declined oxidative stress hepatic markers levels of DEN-induced animals together with vernodalin exposed the radical

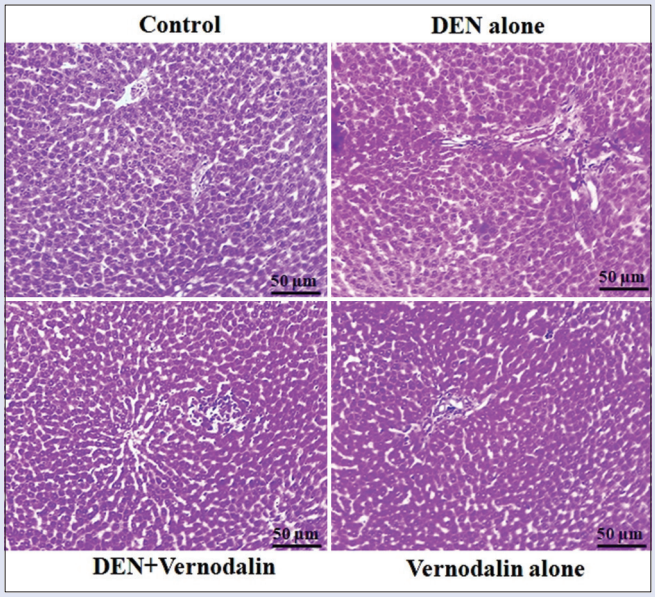

Figure 5: Effect of vernodalin on liver histopathology in control and experimental rats. Normal rats displaying regular liver histology. DEN-induced hepatic tissue presenting vascular channels, nodules, abnormal arrangements, irregular nuclei, and fat deposits. DEN + Vernodalin treated rats hepatic tissue observing only mild alterations. Normal hepatic histology showing in vernodalin alone treated group (Stained with H & E, magnification x40)

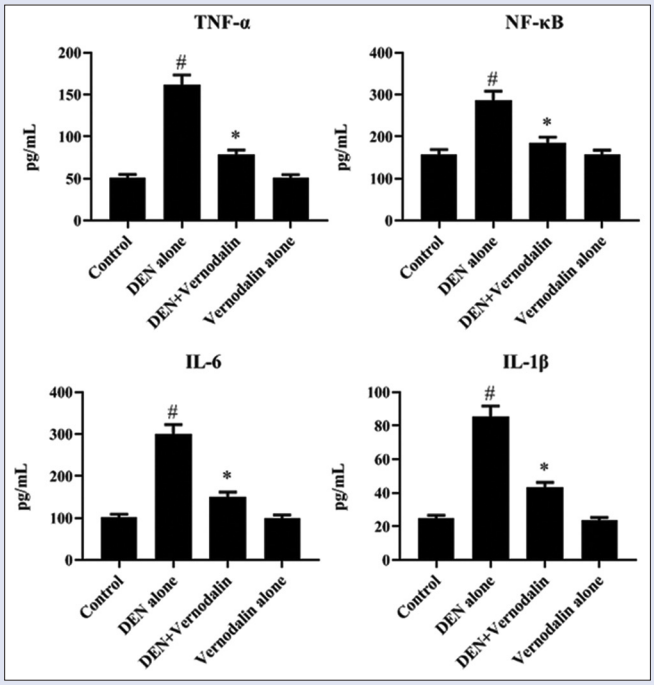

Figure 6: Effect of vernodalin on the levels of cytokines in serum of control and experimental rats. Results were stated as mean ± SD of six observations. #, *$P < 0.05$ indicates the values not sharing a common superscript deliberated as significance

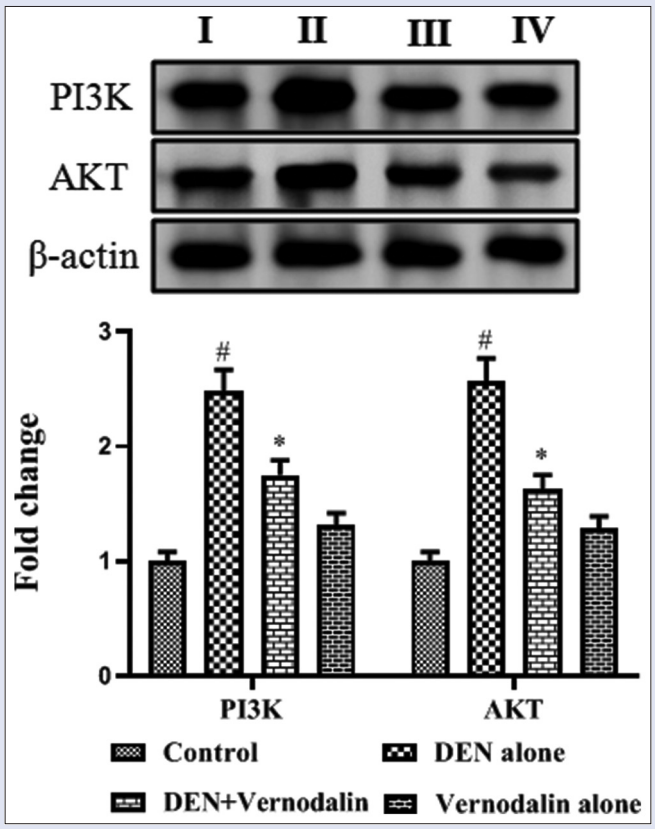

Figure 7: Effect of vernodalin on P13K/AKT signaling pathway in control and experimental rats. Results were stated as mean ± SD of six observations. #, * $P < 0.05$ indicates the values not sharing a common superscript deliberated as significance

scavenging activity. Histopathological observation of DEN-induced rats exhibited the degeneration and loss of architecture in counter to control. Several studies have documented that the liver is a crucial centre for the creation of pro-inflammatory cytokines, which pass through lymphocytes and monocytes accompanied by Kuffer cells. DEN reveals the waves on the Kuffer cells mediated triggering of NF-κB; which induces liver cancer.[46] Vernodalin could reduce the inflammatory mediators in DEN-induced HCC in rats. Remarkably, vernodalin was established to guard counter to DEN-made HCC by aiming the PI3K/Akt pathway as it repressed PI3K and Akt proteins. Hence, our findings suggested that verolidine has been a protective effect against DEN induced liver cancer, and confirmed that it is a good chemotherapeutic drug for future studies.

CONCLUSION

In summary, the upshots of our current research demonstrated that vernodalin employs as a chemopreventive substance versus DEN-induced HCC in rats. The vernodalin possesses antioxidant higher elevation, potent hepatoprotective, anti-inflammatory, apoptotic induction, and anti-cancer activities. Vernodalin was successfully protected from HCC via its action of cell proliferation and apoptotic induction by the regulation of marker PI3K/Akt apoptotic signaling. Hence, vernodalin can be used as a therapeutic material for the treatment of hepatic malignancy.

Authors' contributions

Yifei Guo and Xuan li Li conceived and designed the study; Yao Qu and Lei Yan performed the experiments and writing original manuscript; Shengping Lei, Chenguang Yang analyzed the review and editing; All authors read and approved the final manuscript.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Li S, Yao J, Xie M, Liu Y, Zheng M. Exosomal miRNAs in hepatocellular carcinoma development and clinical responses. J Hematol Oncol 2018;11:54.
2. Balogh J, Victor D, Asham EH, Burroughs SG, Boktour M, Saharia A, *et al.* Hepatocellular carcinoma: A review. J Hepatocell Carcinoma 2016;3:41-53.
3. Farazi PA, DePinho RA. Hepatocellular carcinoma pathogenesis: From genes to environment. Nat Rev Cancer 2006;6:674-87.
4. Liao DJ, Blanck A, Eneroth P, Gustafsson JA, Hällström IP. Diethylnitrosamine causes pituitary damage, disturbs hormone levels, and reduces sexual dimorphism of certain liver functions in the rat. Environ Health Perspect 2001;109:943-7.
5. Park JE, Seo JE, Lee JY, Kwon H. Distribution of seven N-nitrosamines in food. Toxicol Res 2015;31:279-88.
6. Ghosh A, Ghosh D, Sarkar S, Mandal AK, Thakur Choudhury S, Das N. Anticarcinogenic activity of nanoencapsulated quercetin in combating diethylnitrosamine-induced hepatocarcinoma in rats. Eur J Cancer Prev 2012;21:32-41.
7. Heindryckx F, Colle I, Van Vlierberghe H. Experimental mouse models for hepatocellular carcinoma research. Int J Exp Pathol 2009;90:367-86.
8. Kumar A, Jha S, Pattanayak SP. Daphnetin ameliorates 7,12-dimethylbenz[a] anthracene-induced mammary carcinogenesis through Nrf-2-Keap1 and NF-κB pathways. Biomed Pharmacother 2016;82:439-48.
9. Nishikawa T, Nakajima T, Katagishi T, Okada Y, Jo M, Kagawa K, *et al.* Oxidative stress may enhance the malignant potential of human hepatocellular carcinoma by telomerase activation. Liver Int 2009;29:846-56.
10. Jo M, Nishikawa T, Nakajima T, Okada Y, Yamaguchi K, Mitsuyoshi H, *et al.* Oxidative stress is closely associated with tumor angiogenesis of hepatocellular carcinoma. J Gastroenterol 2011;46:809-21.
11. Ding YF, Wu ZH, Wei YJ, Shu L, Peng YR. Hepatic inflammation-fibrosis-cancer axis in the rat hepatocellular carcinoma induced by diethylnitrosamine. J Cancer Res Clin Oncol 2017;143:821-34.
12. Tolba R, Kraus T, Liedtke C, Schwarz M, Weiskirchen R. Diethylnitrosamine (DEN)-induced carcinogenic liver injury in mice. Lab Anim 2015;49 (1 Suppl):59-69.
13. Vijayalakshmi A, Sindhu G. Dose responsive efficacy of umbelliferone on lipid peroxidation, anti-oxidant, and xenobiotic metabolism in DMBA-induced oral carcinogenesis. Biomed Pharmacother 2017;88:852-62.
14. Velu P, Vijayalakshmi A, Iyappan P, Indumathi D. Evaluation of antioxidant and stabilizing lipid peroxidation nature of Solanum xanthocarpum leaves in experimentally diethylnitrosamine induced hepatocellular carcinogenesis. Biomed Pharmacother 2016;84:430-7.
15. De Moura DF, Rocha TA, De Melo Barros D, Da Silva MM, Dos Santos Santana M, Neta BM, *et al.* Evaluation of the antioxidant, antibacterial, and antibiofilm activity of the sesquiterpene nerolidol. Arch Microbiol 2021;203:4303-11.
16. AIMA BAK, Karim A, Sahh M. Antimicrobial activity of certain Sudanese plants used in folkloric medicine. Screening for antibacterial activity. Fitoterapia 1988;59:57-62.
17. Kasim LS, Ferro V, Odukoya OA, Ukpo GE, Seidel V, Gray AI, *et al.* Cytotoxicity of isolated compounds from the extracts of Struchium sparganophora (Linn) Ktze Asteraceae. Pak J Pharm Sci 2011;24:475-8.
18. Pratheeshkumar P, Kuttan G. Vernolide-A, a sesquiterpene lactone from Vernonia cinerea, induces apoptosis in B16F-10 melanoma cells by modulating p53 and caspase-3 gene expressions and regulating NF-κB-mediated bcl-2 activation. Drug Chem Toxicol 2011;34:261-70.
19. Nguyen NH, Nguyen MT, Little PJ, Do AT, Tran PT, Vo XN, *et al.* Vernolide-A and Vernodaline: Sesquiterpene lactones with cytotoxicity against cancer. J Environ Pathol Toxicol Oncol 2020;39:299-308.
20. Looi CY, Arya A, Cheah FK, Muharram B, Leong KH, Mohamad K, *et al.* Induction of apoptosis in human breast cancer cells via caspase pathway by vernodalin isolated from Centratherum anthelminticum (L.) seeds. PLoS One 2013;8:e56643.
21. Sivaramakrishnan V, Shilpa PN, Praveen Kumar VR, Niranjali Devaraj S. Attenuation of N-nitrosodiethylamine-induced hepatocellular carcinogenesis by a novel flavonol-Morin. Chem Biol Interact 2008;171:79-88.
22. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984;21:130-2.
23. Takahara S, Hamilton HB, Neel JV, Kobara TY, Ogura Y, Nishimura ET. Hypocatalasemia: A new genetic carrier state. J Clin Invest 1960;39:610-9.
24. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science 1973;179:588-90.
25. Carlberg I, Mannervik B. Glutathione reductase. Methods Enzymol 1985;113:484-90.
26. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979;582:67-78.
27. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
28. Jiang ZY, Hunt JV, Wolff SP. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal Biochem 1992;202:384-9.
29. Rao KS, Recknagel RO. Early onset of lipoperoxidation in rat liver after carbon tetrachloride administration. Exp Mol Pathol 1968;9:271-8.
30. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
31. Sahani S. Evaluation of hepatoprotective efficacy of APCL-A polyherbal formulation *in vivo* in rats. Ind Drugs 1998;36:628-32.
32. Liu Y, Liu M, Li B, Zhao JL, Zhang CP, Lin LQ, *et al.* Fresh raspberry phytochemical extract inhibits hepatic lesion in a Wistar rat model. Nutr Metab (Lond) 2010;7:84.
33. Dorai T, Aggarwal BB. Role of chemopreventive agents in cancer therapy. Cancer Lett 2004;215:129-40.
34. Lowe SW, Lin AW. Apoptosis in cancer. Carcinogenesis 2000;21:485-95.
35. Rabe T, Mullholland D, van Staden J. Isolation and identification of antibacterial compounds from Vernonia colorata leaves. J Ethnopharmacol 2002;80:91-4.
36. Pedersen MM, Chukwujekwu JC, Lategan CA, Staden Jv, Smith PJ, Staerk D. Antimalarial sesquiterpene lactones from Distephanus angulifolius. Phytochemistry 2009;70:601-7.
37. Koshimizu K, Ohigashi H, Huffman MA. Use of Vernonia amygdalina by wild chimpanzee: Possible roles of its bitter and related constituents. Physiol Behav 1994;56:1209-16.
38. Kupchan SM, Hemingway RJ, Karim A, Werner D. Tumor inhibitors. XLVII. Vernodalin and vernomygdin, two new cytotoxic sesquiterpene lactones from Vernonia amygdalina Del. J Org Chem 1969;34:3908-11.
39. McGurk KA, Brierley CH, Burchell B. Drug glucuronidation by human renal UDP-glucuronosyltransferases. Biochem Pharmacol 1998;55:1005-12.
40. Sallie R, Tredger JM, Williams R. Drugs and the liver. Part 1: Testing liver function. Biopharm Drug Dispos 1991;12:251-9.
41. Erejuwa OO, Sulaiman SA, Wahab MS, Sirajudeen KNS, Salleh MS, Gurtu S. Hepatoprotective effect of tualang honey supplementation in streptozotocin-induced diabetic rats. Int J Appl Res Nat Prod 2011;463:39-44.
42. Parola M, Robino G. Oxidative stress-related molecules and liver fibrosis. J Hepatol 2001;35:297-306.
43. Hirose M, Imaida K, HOCTTS. Cancer Chemoprevention by Antioxidants, Food Phyto Chemicals: Teas, Spices and Herbs. Washington: American Chemical Communications Society Press; 2016. p. 122-32.
44. Kamaraj S, Vinodhkumar R, Anandakumar P, Jagan S, Ramakrishnan G, Devaki T. The effects of quercetin on antioxidant status and tumor markers in the lung and serum of mice treated with benzo (a) pyrene. Biol Pharm Bull 2007;30:2268-73.
45. Thirunavukkarasu C, Singh JP, Selvendiran K, Sakthisekaran D. Chemopreventive efficacy of selenium against N-nitrosodiethylamine-induced hepatoma in albino rats. Cell Biochem Funct 2001;19:265-71.
46. Seki E, Brenner DA. Toll-like receptors and adaptor molecules in liver disease: Update. Hepatology 2008;48:322-35.