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Review Article

Decoding DEN-Induced Hepatocellular Carcinoma: Models, Mechanisms, and Ameliorative Strategies in Preclinical Research

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Abstract

Objective: This review focuses specifically on the DEN-induced hepatocellular carcinoma model, with the objectives are to describing the establishment and characterisation of the DEN model, delineating the underlying molecular and cellular mechanisms driving DEN-induced hepatocarcinogenesis, and exploring various ameliorative treatment strategies investigated, including natural products and AgNPs, along with their proposed mechanisms of action. The review also discusses the translational relevance and limitations of the DEN model and outlines future directions in preclinical HCC research aiming to improve therapeutic outcomes.

Data sources: DEN, HCC, and hepatocarcinogenesis were among the search terms used to search databases such as PubMed, Google Scholar, and ScienceDirect. After screening abstracts and titles, conference proceedings and commentary were not included. 95 articles and book chapters were included in the final analysis out of 137 sources.

Summary: Future preclinical HCC studies employing DEN models will concentrate on overcoming constraints by combining therapies, targeting tumour heterogeneity, and leveraging enhanced drug delivery technologies, dietary restrictions, tumor microenvironment, and multi-omics approaches. Treatment results are improved by the focused, low-toxicity administration of anti-HCC medicines made possible by nanoparticles. Multi-omics technology integration can reveal new therapeutic targets, biomarkers, and mechanisms influencing the course of HCC and the effectiveness of treatment.

Conclusion: Because it mimics important aspects of actual HCC, the DEN-induced HCC model has proven essential in studying the course of liver cancer and testing treatments. The model is nevertheless crucial for investigating therapies, particularly in light of developments in omics and nanotechnology that are bringing preclinical findings closer to clinical use.

Keywords: DEN (diethylnitrosamine), HCC (hepatocellular carcinoma), CCl₄ (carbon tetrachloride), Hepatocarcinogenesis, AgNPs.

1. Introduction

Hepatocellular carcinoma (HCC) is the most common kind of primary liver cancer and one of the leading causes of death worldwide. Its global prevalence is increasing due to aetiologies such as hepatitis B and C infection, aflatoxin exposure, excessive alcohol use, non-alcoholic fatty liver disease (NAFLD), obesity, and metabolic syndrome. HCC often occurs in chronic liver disease and cirrhosis, preventing early detection and treatment.¹⁻⁴ Although surgical resection and liver transplantation can cure early-stage HCC, they are not recommended for advanced-stage patients. Systemic treatments, such as sorafenib and cisplatin, have proven ineffectual due to tumour resistance and heterogeneity. As a result, the case necessitates new therapeutic approaches as well as a better knowledge of illness mechanisms.⁵⁻⁸

Human hepatocarcinogenesis research is difficult due to disease heterogeneity, extended latent periods, and ethical problems; hence, preclinical animal models are critical for HCC research.^{8,9} Chemically induced models containing N-nitrosodiethylamine/diethylnitrosamine (DEN) are recommended because of their convenience, reproducibility, and ability to replicate the multistage course of human HCC, which is accompanied by liver damage and fibrosis. These models enable mechanistic investigation and preclinical testing of medicinal molecules, including natural products and nanoparticles. The current review focuses on the DEN-induced HCC model and its utilisation with cell lines, intending to understand its molecular underpinnings, therapeutic studies, translational implications, and inherent constraints in preclinical HCC research. HCC is a heterogeneous malignancy that is usually associated

with chronic liver damage and fibrosis, resulting in a wide range of clinical and histological characteristics.^{4,9,10}

Its pathogenesis is a multistep process marked by genetic mutations, epigenetic changes, oxidative stress, chronic inflammation, and immune dysregulation. HCC is morphologically heterogeneous from well- to poor-differentiation tumours, with subtypes such as fibrolamellar HCC showing distinct characteristics and prognosis. Molecular profiling has shown similar changes in pathways such as Wnt/ β -catenin, PI3K/Akt, JAK/STAT, MAPK, and p53.^{9,11-14} The tumour microenvironment, which includes immune cells, hepatic stellate cells, and hypoxia-induced oxidative stress, also promotes cancer growth, metastasis, and treatment resistance. The DEN-induced HCC model has been widely used in preclinical research because of its repeatability and capacity to replicate stepwise hepatocarcinogenesis, from acute liver injury to established tumours.^{10,11,15,16}

Such a model is economical and particularly useful when used in combination with agents such as carbon tetrachloride (CCl₄) to mimic fibrosis, which closely mimics human HCC related to chronic liver disease. The DEN model has its drawbacks, however, since it primarily models chemical carcinogenesis and might not completely reflect viral (HBV, HCV) or NAFLD-related HCC subtypes. In addition, DEN-induced tumors are characteristically found to have a diffuse distribution in the liver as opposed to the solitary lesions commonly found in human infections, and rodent species-specific immune response can affect translational significance for immunotherapy research.^{8,12,16,17} In comparison with other models, including genetically modified mouse models (GEMMs), diet models, and patient-derived xenografts (PDXs), DEN has a less complicated strategy for investigating tumour development and initiation. GEMMs can replicate genetic causes more accurately but are technically challenging, while orthotopic and xenograft models suit the assessment of response to therapy but do not provide the natural context of tumour progression that DEN does. Therefore, with all its shortcomings, the DEN model continues to be an important workhorse in hepatocarcinogenesis studies, especially when investigating the interaction between liver damage, fibrosis, and cancer onset.^{13,18}

2. Methodology

For this study, various databases were used, including Google Scholar, PubMed, MDPI, Web of Science, Sage, Science Direct journals, etc. The terms used were N-nitrosodiethylamine/diethylnitrosamine/DEN, Hepatocellular carcinoma/HCC, hepatocarcinogenesis, Carbon tetrachloride/CCl₄, liver malignancy, pre-HCC, signalling pathway, etc. Studies were chosen for first evaluation, and titles and abstracts were evaluated for eligibility as research or review articles/books/book chapters, omitting conference proceedings and commentary. From 137 papers, 95 articles/books/book chapters were deemed to be eligible and selected for the final analysis.

3. DEN-Induced Hepatocarcinogenesis: Model Establishment and Characterisation

Induction of the DEN-induced HCC model in rodents requires well-defined protocols for dosage, duration, and route of DEN administration. These characteristics are crucial in determining cancer latency, incidence, and histology. In mice, one frequent way is to administer DEN intraperitoneally (IP) at 10-100 mg/kg body weight in 2-week-old mice, targeting proliferative hepatocytes and successfully beginning tumorigenesis. Alternatively, low-dose recurrent exposure, whether via IP injection or drinking water, mimics chronic carcinogen exposure and is used to research cancer promotion and progression. Co-administration of tumour promoters like CCl₄ strongly promotes HCC development by simulating chronic liver damage and fibrosis and thereby increasing the translational value of the model to human HCC pathogenesis. The delivery route of DEN also plays a role: IP injection guarantees precise dosing for initiation studies, while oral routes simulate environmental exposure but can affect outcomes through interactions with the gut-liver axis.^{10,19}

Susceptibility of species and strain is significant in model design. Mice, especially strains such as C57BL/6, are hypersensitive to DEN compared to rats, with increased tumour frequency and shorter latent periods. Males are more susceptible than females due to sex hormone effects on hepatic metabolism and inflammatory response.²⁰ The DEN/CCl₄ model is widely employed to mimic the fibrosis-related liver microenvironment characteristic of human HCC developing from hepatitis or NAFLD. CCl₄ causes liver injury through Cytochrome P450-2E1 (CYP2E1)-dependent production of reactive metabolites, leading to lipid peroxidation, oxidative stress, inflammation, and accumulation of extracellular matrix.¹⁰ The DEN is an initiator by inducing genetic mutations, and CCl₄ induces clonal growth of mutated hepatocytes in a regenerative environment. Such a combined effect has been effectively utilised to screen for agents such as Isorhamnetin and silver nanoparticles to evaluate their therapeutic efficacy.^{11,21}

Assessment of DEN models of HCC includes a multi-faceted strategy. Biochemical assays for ALT (Alanine Transaminase), AST (Aspartate Transferase), ALP (Alkaline Phosphatase), and GGT (Gamma-Glutamyl Transferase) determine liver damage and function. AFP (Alpha-Fetoprotein) is the most important tumour marker in DEN models, but it is poorly sensitive to early or well-differentiated tumours.^{18,22} Histopathological examination with haematoxylin and eosin evaluates tumour grade and type, and fibrosis examination employs Masson's trichrome or picosirius red staining, especially in DEN/CCl₄ models.¹⁰ A progression of distinct histopathological changes in the liver characterises DEN-induced hepatocarcinogenesis. Early changes include hepatocyte degeneration, necrosis, inflammation, and bile duct proliferation.²³ This is followed by the formation of dysplastic nodules, comprising both low-grade and high-grade lesions, which signify advancing liver pathology. Ultimately, the

process culminates in the development of HCC, characterised by well-defined nodules containing atypical hepatocytes.²⁴⁻²⁷

Immunohistochemical labelling detects cell proliferation (e.g., Ki-67), apoptosis (e.g., cleaved caspase-3), and particular markers (e.g., CK19 or STAT3). Molecular analysis, including qPCR, Western blotting, and ELISA, studies gene and protein expression in pathways such as PI3K/Akt, Wnt/ β -catenin, NF- κ B, and JAK/STAT.^{11-14,21,28} Microarray or RNA-sequencing analysis produces transcriptome data, whereas DNA methylation and histone modification studies identify epigenetic regulation. Ultrasound, MRI, and bioluminescence imaging (BLI) are imaging modalities that allow for longitudinal evaluation of cancer progression and treatment.^{12,29-31} In vitro investigations in human and animal liver cancer cell lines such as HepG2, Huh7, MHCC97-H, and PLC/PRF/5 supplement in vivo data.^{30,31} Viability, apoptosis, and migration experiments in in vitro models allow for a thorough analysis of cell processes, drug sensitivity, and pathway alterations. Phyllanthin and Cryptotanshinone, for instance, exhibited anticancer activity in DEN-treated animals and in vitro in HepG2 or Huh7 cells. This combined strategy improves the mechanistic insight and translational significance of preclinical HCC studies.^{21,32,33}

4. Molecular and Cellular Mechanisms of DEN-Induced HCC

4.1 Metabolic Activation and Genotoxicity of DEN

DEN is a typical procarcinogen that must be metabolically activated to cause genotoxicity and carcinogenesis. When exposed to DEN, molecular and cellular changes occur, leading to uncontrolled proliferation, increased survival, and tumorigenesis. DEN is mostly activated in the liver, producing reactive intermediates that bind to DNA and proteins.³⁴ CYP2E1, primarily found in hepatocytes, is the most active enzyme in DEN activation. It catalyses the α -hydroxylation of DEN, which is a crucial step for its carcinogenic metabolites. Although other CYP enzymes are involved, CYP2E1 is the main isoform. Its expression level has a considerable impact on DEN-induced hepatotoxicity and tumorigenesis risk. CYP2E1 inducers and inhibitors can influence DEN bioactivation and carcinogenic activity. After α -hydroxylation, DEN is transformed to α -hydroxynitrosamine, which degrades to acetaldehyde and ethyl diazonium ions.³⁵

4.2 DNA Damage and Mutagenesis

The ethyl diazonium ion, a strong electrophile, assaults nucleophilic sites in DNA, RNA, and proteins, causing mutagenesis events that lead to cancer. DEN metabolism reactive metabolites alkylate and oxidise DNA, causing damage. The ethyl diazonium ion ethylates DNA bases, producing adducts such as O⁶-ethylguanine, N⁷-ethylguanine, and O⁴-ethylthymine. O⁶-ethylguanine is highly mutagenic as it mispairs with thymine during DNA replication, leading to G:C to A:T transitions, which are prevalent in oncogenes and tumour suppressor genes. N⁷-ethylguanine and O⁴-ethylthymine are less

mutagenic and more easily repaired. Besides direct base alterations, DEN metabolites are also responsible for DNA strand breaks, which can occur during the repair of alkylated bases or as a result of enhanced oxidative stress. Oxidative lesions 8-oxo-2'-deoxyguanine (8-oxo-dG) weaken the integrity of DNA and enhance chromosomal instability, a characteristic of cancer. Cells counteract DEN-induced damage by employing a variety of repair processes. Base excision repair (BER) is mostly concerned with fixing N-alkylated and oxidised bases, while O⁶-ethylguanine damage is reversed directly by O⁶-alkylguanine-DNA alkyltransferase (AGT or MGMT), a suicide enzyme. However, excessive DEN exposure depletes AGT, leaving cells susceptible to mutagenesis. In states of hyper DNA damage or accelerated proliferation, like in models of chronic liver injury, translesion synthesis or error-prone repair can take place, leading to fixation of mutation and genomic instability.³⁶⁻³⁸

4.3 Oxidative Stress and Reactive Oxygen/Nitrogen Species (ROS/RNS)

DEN markedly elevates oxidative stress in the liver, which leads to hepatocellular damage, inflammation, and cancer induction. CYP2E1-catalysed metabolism of DEN produces superoxide radicals through enzyme uncoupling. DEN- and co-carcinogen-induced inflammatory responses mobilise immune cells (e.g., Kupffer cells and neutrophils) that produce ROS and RNS such as NO \bullet and ONOO $^-$. Furthermore, mitochondrial dysfunction in DEN-induced hepatotoxicity is a second source of intracellular ROS.^{39,40} Overproduction of ROS/RNS induces lipid peroxidation, which produces aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), which interact with proteins and nucleic acids and damage their function. Mutation and genomic instability are generated by oxidative DNA damage, specifically 8-oxo-dG. DEN exposure can disrupt the antioxidant defence system, which comprises enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as non-enzymatic antioxidants including glutathione (GSH), vitamin C, and vitamin E.⁴¹ This type of imbalance increases oxidative stress, which leads to DNA damage, chronic inflammation, and the development of liver tumours. In DEN-induced models, the majority of chemopreventive research focuses on drugs that restore redox equilibrium.

4.4 Inflammation and Immune Cell Infiltration

Chronic inflammation is a hallmark of cancer and plays a significant role in the formation of HCC, especially in DEN models, in conjunction with drugs like CCl₄, which generate chronic liver injury. Kupffer cells, the liver's resident macrophages, contribute significantly to inflammation after exposure to hepatotoxic substances such as DEN and CCl₄. These macrophages cause hepatocellular injury and generate damage-associated molecular patterns (DAMPs), which stimulate Kupffer cells. Activated Kupffer cells release pro-inflammatory cytokines and chemokines, such as TNF- α , IL-6, and IL-1 β , leading to inflammation, immune cell recruitment, and hepatocyte proliferation. Kupffer cell mediators and hepatocellular injury attract immune cells

such as neutrophils, monocytes, lymphocytes (T and B cells), and NK cells to the liver.^{16,34} While some immunity subgroups improve anti-tumor immunity, chronic inflammation causes immunosuppressive and tumor-promoting populations such as TAMs and MDSCs, resulting in immune evasion and tumour progression. TNF- α and IL-6, pro-inflammatory cytokines, play a key role in hepatocarcinogenesis. TNF- α activates the NF- κ B pathway, promoting cell proliferation and survival, while IL-6 activates the JAK/STAT3 pathway, which promotes hepatocyte proliferation, suppresses apoptosis, and promotes tumour growth.^{13,16} Chemokines direct inflammatory cells to injury sites and tumours, promoting a pro-tumorigenic environment. Chronic inflammation helps tumours through a variety of mechanisms: chronic ROS/RNS cause DNA damage; cytokines and growth factors stimulate hepatocyte proliferation, increasing the likelihood of mutations; and the inflammatory environment promotes angiogenesis, invasion, and metastasis. Chronic inflammation, oxidative stress, and DNA damage are key factors in DEN-induced hepatocarcinogenesis, especially with CCl₄ co-treatment that promotes liver regeneration. In these models, therapeutic methods frequently focus on inhibiting inflammatory signalling and lowering pro-inflammatory mediators.^{11,13,42}

4.5 Cell Cycle Control Dysregulation

Unregulated hepatocyte proliferation is one of the most important aspects of DEN-induced hepatocarcinogenesis, and it is most closely related to cell cycle disruption. Cyclins and cyclin-dependent kinases (CDKs) regulate cell cycle progression. Cyclin D1-CDK4 and Cyclin E-CDK2 complexes control the G1/S transition by phosphorylating and inhibiting the retinoblastoma protein (pRb), hence releasing E2F transcription factors required for S-phase entry. In most cases of DEN-induced HCC, critical G1/S-phase regulators—Cyclin D1, Cyclin E, CDK2, and CDK4—are overexpressed, resulting in premature and uncontrolled entry into DNA synthesis and cell division. Under normal conditions, DNA damage activates the checkpoint proteins p53 and p21 (CDKN1A), which stop the cell cycle and allow repair or trigger apoptosis. The G2/M checkpoint also inhibits mitosis when there is damaged or unreplicated DNA. But in DEN-induced HCC, tumour suppressor gene mutations or epigenetic silencing of genes such as TP53 may inactivate these checkpoints and enable proliferation of genetically unstable hepatocytes. The p16(INK4a)/Rb pathway is also frequently inactivated, further compromising cell cycle control. The overexpression of cyclins/CDKs in conjunction with the inactivation of checkpoints leads to hyperplasia of hepatocytes. This state not only defines malignant transformation but also allows the stacking of additional genetic and epigenetic changes. In DEN/CCl₄ models, CCl₄-induced liver injury stimulates regeneration, again stimulating cell division and tumour advancement.¹¹⁻¹³ Thus, the targeting of cell cycle deregulation is a prime strategy in therapeutic therapy for HCC.

5. Key Signalling Pathways in DEN-Induced HCC

DEN-induced hepatocarcinogenesis is driven by the aberrant activation or inactivation of various signalling pathways that regulate cell growth, survival, proliferation, and differentiation. Several key pathways have been implicated.

5.1 PI3K/Akt Pathway Activation

The PI3K/Akt signalling pathway is a major pro-survival and anti-apoptotic pathway that is frequently constitutively activated in human HCC and DEN models. This route promotes cell growth, proliferation, and survival while inhibiting apoptosis. Mutations in pathway elements (e.g., PIK3CA, PTEN) can cause activation, as can upstream signals from growth factor or inflammatory cytokine receptors. In rats, hesperidin inhibits the PI3K/Akt pathway, preventing it from protecting against chemically induced liver cancer. Cryptotanshinone inhibits the PI3K/Akt/mTOR signalling pathway in HCC cells and xenografts.^{11,13,21,43}

5.2 Wnt/beta-Catenin Signalling

The Wnt/beta-Catenin pathway is commonly deregulated in human HCC by mutations in CTNNB1 (beta-Catenin) or AXIN1. In DEN models, tumorigenesis is also caused by the activation of this pathway. Activated Wnt signalling stabilises and translocates beta-Catenin to the nucleus, where it functions as a co-transcription factor for proliferation genes (e.g., Cyclin D1, c-Myc). Dysregulation of Wnt/beta-Catenin signalling can enhance the survival and growth of initiated cells.^{11,12,14}

5.3 MAPK Pathways (ERK, JNK, p38)

Mitogen-Activated Protein Kinase (MAPK) cascades, such as ERK, JNK, and p38, transmit cell surface receptor signals to the nucleus and regulate a variety of cellular processes, including proliferation, differentiation, stress responses, and apoptosis. In DEN-induced HCC, the activity of these pathways can be modified. For instance, the ERK pathway is frequently implicated in cell proliferation and survival. JNK and p38 pathways are generally induced by stress signals and can mediate pro-apoptotic or pro-survival responses depending on context. Dysregulation or inappropriate activation of MAPK pathways is involved in the uncontrolled survival and growth of tumour cells.⁴⁴

5.4 Other Relevant Pathways (e.g., STAT3, NF- κ B)

Other signalling pathways are activated during DEN-induced hepatocarcinogenesis. IL-6 and other cytokines in the inflammatory environment also activate the STAT3 pathway, which increases cell proliferation, survival, and angiogenesis. Inflammatory stimuli, including TNF- α and stress, activate NF- κ B, a transcription factor that promotes cell survival and proliferation. The IGF signalling pathway, notably IGF2, is also involved in the development of liver tumours and can be addressed therapeutically. TGF-beta signalling dysregulation, with both tumour-suppressive and pro-tumorigenic functions depending on the stage, is also pertinent. The complex crosstalk among these pathways, under the control of genetic changes, epigenetic changes,

oxidative stress, and inflammation, impels the process from an initiated cell to a cancerous tumour.^{11,13,14,45}

5.5 Epigenetic Changes in DEN-Induced Hepatocarcinogenesis

In addition to genetic mutations, epigenetic alterations—heritable gene expression modifications that do not involve changes to the underlying DNA sequence—contribute to DEN-induced HCC development. Methylation of DNA, or the conversion of methyl groups to cytosine on CpG dinucleotides, is a fundamental epigenetic regulatory mechanism. Global hypomethylation and regional hypermethylation of CpG island-containing promoter regions are common in cancer. Global hypomethylation may cause oncogene activation or chromosomal instability, whereas hypermethylation of tumour suppressor gene promoters silences them. In DEN-induced HCC, abnormal methylation patterns were observed, including the silencing of cell cycle regulators, proapoptotic genes, and signalling pathway genes. For example, demethylation of the foetal IGF2 promoter causes overexpression, which has also been found in subpopulations of human HCC and may apply to DEN models.^{45,46} Histone proteins undergo numerous post-translational changes, including acetylation, methylation, phosphorylation, and ubiquitination, which regulate chromatin shape and gene transcription. These alterations are controlled by enzymes such as histone acetyltransferases (HATs), histone deacetylases (HDACs), methyltransferases, and demethylases, the majority of which are overexpressed in cancer. In DEN-induced hepatocarcinogenesis, abnormal histone modification patterns most likely alter normal gene expression, increasing oncogene activation and suppressing tumour suppressor genes.⁴⁶

Such alterations favour hepatocyte proliferation, survival, and EMT, all of which are required for metastasis. MicroRNAs (miRNAs) are short non-coding RNAs that regulate gene expression by binding to target mRNA's 3' untranslated regions (UTRs), causing destruction or translational repression. Abnormal miRNA expression is prevalent in HCC and DEN-induced models. MiRNAs are either tumour suppressors or oncomiRs (oncogenes). Reduced tumor-suppressive miRNAs or higher oncomiRs in DEN-induced HCC may cause enhanced cell proliferation, apoptosis resistance, invasiveness, and treatment resistance. Certain oncogenic or tumor-suppressive miRNAs have been identified in DEN models. Circ-PRKCI, for example, functions as a competitive endogenous RNA (ceRNA) by binding to miR-545. Such binding decreases miR-545 expression, raising its target gene AKT3, promoting HCC tumour growth.⁴⁶⁻⁴⁸

6. Mechanisms of Action

6.1 Natural Products as Chemopreventive and Therapeutic Agents in DEN-Induced Hepatocarcinogenesis

Plant-derived natural goods, marine-derived products, and other natural biological sources have also received attention as potential chemopreventive or therapeutic agents against HCC, particularly in DEN-

induced experimental models. These natural chemicals have a pleiotropic effect, meaning they modulate multiple cancer-related pathways simultaneously. Most natural compounds have a high antioxidant activity, which allows them to effectively scavenge ROS and RNS, hence lowering oxidative stress. Oxidative damage is a crucial element in DEN-induced hepatocarcinogenesis, which can be increased by co-carcinogens like CCl₄. Several natural products, such as *Tetilla dactyloidea*, *Averrhoa carambola*, *Terminalia chebula*, and *Cucumis melo* extracts, have demonstrated significant antioxidant activity by restoring endogenous antioxidant enzyme levels (e.g., SOD, CAT, GPx) and lowering lipid peroxidation markers (e.g., MDA), thus reducing DNA damage and inflammation. Chronic inflammation plays a key role in tumour development and progression in DEN models. Natural products exhibit anti-inflammatory properties, which reduce inflammatory cell activation, downregulate pro-inflammatory cytokines such as TNF- α and IL-6, and block key signalling pathways, including NF- κ B.^{26,49-51}

Cancer cells are resistant to apoptosis. Natural substances overcome this resistance by reactivating programmed cell death pathways in HCC cells. This is accomplished by the activation of intrinsic apoptotic pathways involving caspases 3 and 9, modulation of the Bax/Bcl-2 ratio, and disruption of survival signals.^{21,52} Compounds such as nanocurcumin, phyllanthin, isorhamnetin, hesperidin and cryptotanshinone, for example, have been shown to induce apoptosis in HCC models by activating caspases and permeabilising the mitochondrial membrane. Some natural compounds reduce proliferation by influencing cell cycle regulators.^{21,32,33,53-55} The activity includes cyclin downregulation (e.g., Cyclin D1, Cyclin E), CDK inhibition, and activation of CDK inhibitors, including p21 and p27.^{21,52} Natural substances use similar movements to halt the cell cycle at important checkpoints (usually G1 or G2), preventing excessive cell proliferation and cancer formation. The anti-proliferative action of such drugs is an important field of study in DEN model research. Natural compounds also inhibit dysregulated signalling pathways, which lead to HCC development. Luo *et al.* (2020) discovered that the PI3K/Akt, Wnt/ β -catenin, and MAPK pathways control cell proliferation, survival, and invasion. Hesperidin and cryptotanshinone, for example, have been demonstrated to block PI3K/Akt signalling in HCC cells, resulting in reduced cell survival and death.^{21,54} Natural compounds can disrupt different parts of cancer biology by altering dominant pathways, making them more effective as multi-targeted therapy.

6.2 Mechanisms of Action of Silver Nanoparticles in DEN-Induced HCC

Nanomaterials are useful in cancer therapy because of their unique features that allow for targeted administration and increased efficacy. Among the numerous nanoparticles, such as polymeric nanoparticles, liposomes, magnetic nanoparticles, gold nanoparticles, selenium nanoparticles, and silver nanoparticles (AgNPs), AgNPs are widely investigated for anti-cancer activity in DEN-induced HCC models.⁵⁶⁻⁶⁰

AgNPs are cytotoxic to cancer cells; they are produced using chemical reduction or green synthesis using plant extracts, which may have bioactive properties. AgNPs are often surface-modified with stabilisers or bioactive compounds like polyvinylpyrrolidone (PVP) to improve biocompatibility, stability, and tumour targeting.^{24,25,27,51,58}

AgNPs can be entrapped in organelles such as mitochondria, inducing cell damage and apoptosis. AgNPs have been shown to reduce liver cancer cell survival and tumour formation in DEN-induced HCC models.^{58,61} AgNPs cause cell death in tumour cells primarily through oxidative stress by boosting reactive oxygen species (ROS) and reactive nitrogen species (RNS), which overwhelm tumour cells' defence mechanisms.⁶⁰ Ag⁺ ions also destabilise the mitochondrial electron transport chain, increasing ROS production. This oxidative lipid, protein, and DNA damage induces death in tumor cells, which are less resilient to such stress than normal cells.⁶² In liver injury models, AgNPs decrease systemic oxidative stress markers and induce stress in tumor cells specifically, indicating protective and therapeutic effects. AgNPs also regulate cell death pathways such as apoptosis and autophagy. AgNP-induced mitochondrial failure activates caspases and modulates Bcl-2 family proteins, hence initiating the intrinsic apoptotic cascade. They enhance pro-apoptotic markers such as Bax and caspase-3 while decreasing anti-apoptotic proteins like Bcl-2. AgNPs also modulate autophagy, which may have dual roles in cancer depending on the situation. AgNPs are pro-apoptotic and pro-survival towards cancer cells. AgNPs also induce cell cycle arrest in G1 through the downregulation of cyclin D1 and CDK2, and inhibit the migration of HCC cells and increase their anti-tumor activity. DEN is a strong liver carcinogen that is primarily metabolised by CYP2E1 to form reactive ethyl diazonium ions.⁶⁰⁻⁶⁵

The electrophilic medications alkylate DNA to form mutagenic adducts like O⁶-ethylguanine that, when not repaired, induce hepatocarcinogenesis. The resulting regenerative proliferation following acute DEN-induced damage serves to correct these mutations, leading to tumor development. Recurrent cell turnover and persistent inflammation also continue to propel tumour growth in a compromised liver microenvironment.^{62,66,67} Some of the cellular pathways affected by DEN are also modulated by AgNPs. In DEN models, PI3K/Akt/mTOR, Wnt/ β -catenin, Ras/MAPK, Hedgehog, and Notch pathways get aberrantly activated, stimulating proliferation, survival, and migration of cancer cells.^{34,68-82} AgNPs can reverse this process by regulating these pathways. They reduce PI3K/Akt signalling, which leads to lower cell survival and proliferation. Furthermore, AgNPs affect cell cycle progression by acting on cyclins and CDKs, namely those that control the G1/S transition. ROS and RNS produced during DEN metabolism have a dual role in hepatocarcinogenesis. Moderate oxidative stress initiates mutation and carcinogenic signalling.^{62,83} ROS activate survival pathways, including PI3K/Akt and NF- κ B, resulting in inflammation, fibrosis, and a tumor-supportive microenvironment. Excess ROS can also

induce cell death by apoptosis or ferroptosis, acting as a tumour inhibitory mechanism. Cancer cells frequently become reliant on increased antioxidant defences (e.g., the Nrf2 pathway), leaving them especially sensitive to therapies that increase oxidative stress or inhibit these defences.^{79,80,84,85}

6.3 Dietary and Lifestyle Interventions

Nutritional and lifestyle determinants play a crucial role in HCC risk, especially in NAFLD.⁸⁴ Preclinical DEN models have investigated whether dietary and lifestyle modifications can prevent or reduce DEN-induced hepatocarcinogenesis. Dietary restriction (DR) decreases caloric intake without malnutrition and is found to increase longevity as well as retard age-related diseases, such as cancer, in most organisms. DEN-induced HCC models have been studied to show that DR is highly protective against tumorigenesis in hepatocellular cells. Duan *et al.* (2017) established that dietary restriction was able to suppress tumour development in DEN-treated mice. The mechanisms by which DR exerts its protection are multifaceted and complex. DR can affect many cellular processes, such as metabolism, stress resistance, DNA repair, as well as control of the cell cycle. In the case of DEN-induced HCC, DR was demonstrated to restore the altered gene expression profile of tumorigenesis to a more normal state. This included controlling genes involved in cell growth, death, inflammation, and metabolism. DR can impact hormonal signalling pathways, such as insulin and IGF-1, which are frequently disturbed in HCC and contribute to tumour growth. By changing the metabolic landscape and amplifying cellular repair processes, DR favours a less permissive environment for DEN-induced tumour initiation and development. This indicates the potential of nutritional interventions, in this case, caloric restriction, as a chemopreventive agent in HCC and calls for further elucidation of the specific molecular targets and translational value in at-risk human beings.^{75,86}

6.4 Other Potential Agents

Cisplatin, sorafenib, and gemcitabine are chemotherapeutic medications whose principal mechanism of action is to generate DNA cross-links, which impede replication and transcription and eventually lead to death in growing cells. Although successful in a wide range of cancers, cisplatin is ineffective as a monotherapy in advanced HCC due to inherent or acquired resistance and systemic toxicity.^{6,7,61,87} In DEN-induced HCC preclinical models, these chemotherapeutic drugs have been tested with various agents combined to identify synergistic anti-cancer activity and to develop strategies to reduce their toxic profile. Of particular interest, the two-fold activity, anticancer synergy and toxicity reduction, renders AgNPs an interesting addition in combination regimens. The pathways through which AgNPs might mitigate side effects are conjectured to include the inhibition of oxidative stress and inflammation in non-target healthy tissues, especially the liver and kidneys, which are most often damaged. Yet these protective interactions are multifaceted and context-sensitive and must be

elucidated through close mechanistic work with well-defined DEN-induced HCC models.^{61,87}

7. Mechanisms Underlying Ameliorative Effects

Ameliorative therapies explored in DEN-induced HCC models impact by regulating more than one molecular pathway by disturbed during hepatocarcinogenesis. Such interventions generally aim at mechanisms associated with oxidative stress, inflammation, apoptosis, cell cycle regulation, and tumour microenvironment disruption.

7.1 Reducing Oxidative Stress

Oxidative stress plays a pivotal role in DEN-induced liver cancer, inducing reactive oxygen (ROS) and nitrogen species (RNS) to inflict DNA, protein, and lipid damage. Ameliorative compounds try to suppress ROS/RNS or stimulate liver antioxidant processes. Most protective compounds boost antioxidant enzymes—such as SOD, CAT, GPx, and glutathione S-transferases (GSTs)—to minimize oxidative stress. Induction is typically through the Nrf2 pathway, activating transcription of ARE-regulated genes on induction.^{62,85,88} *Andrographis paniculata*, *Carissa carandas*, *Madhuca longifolia*, *Crocus sativus*, *Cucumis melo* mediated AgNPs and *Tetilla dactyloidea*, *Averrhoa carambola*, *Terminalia chebula*, and *Cucumis melo* extracts have been shown to elevate antioxidant enzymes and minimise oxidative stress in DEN models. Many natural compounds are also free radical scavengers. Flavonoids and phenolic compounds neutralise free radicals, inhibiting lipid peroxidation and oxidative DNA damage. These effects support endogenous defences, boosting cellular protection against carcinogenic damage.^{24-27,49-51,89}

7.2 Inflammation Regulation

Chronic inflammation creates a tumour-favouring environment, with damage leading to recruitment of immune cells and upregulation of proinflammatory mediators in the DEN model. Cytokines TNF- α , IL-6, and IL-1 β are upregulated in DEN-induced damage in the liver, facilitating survival, proliferation, and recruitment of immune cells. These cytokines are targeted by compounds such as nanocurcumin, hesperidin, and isorhamnetin, which interrupt the pro-tumour cycle. AgNPs synthesised from plant extracts also have anti-inflammatory activity, adding to their chemopreventive efficacy. NF- κ B, a transcription factor that is activated by inflammatory stimuli, ROS, and liver injury, is commonly aberrantly active in HCC. NF- κ B regulates inflammation, proliferation, and survival-related genes. NF- κ B inhibition by compounds such as curcumin, isorhamnetin, or AgNPs represses the expression of proinflammatory genes, typically by inhibiting I κ B degradation or NF- κ B nuclear translocation. NF- κ B inhibition suppresses tumour-favouring inflammation and induces apoptosis.^{53-55,78,79,84}

7.3 Restoring Cell Cycle and Apoptosis Control

DEN-induced HCC results in cell cycle disruption and apoptosis evasion. Curing agents address these problems to restore checkpoints and remove defective hepatocytes. Caspases induce apoptosis, with -3, -7, and -

9 being induced by treatments to induce cancer cell death. Phyllanthin and polydatin induce apoptosis in HCC cell lines by triggering caspases, resulting in tumor inhibition.^{32,33,79} Mitochondrial apoptosis is regulated by the Bcl-2 family, with DEN-induced tumors showing increased anti-apoptotic proteins (Bcl-2, Bcl-xL) and decreased pro-apoptotic ones (Bax, Bak).³⁵ Agents such as polydatin resolve this imbalance by downregulating Bcl-2 and upregulating Bax, increasing apoptosis sensitivity.⁷⁹ Inhibition of the Hedgehog pathway targets Bcl-2/Beclin-1 interactions, increasing autophagy and apoptosis. Dysregulation of the cyclin/CDK system is prevalent in HCC. Overexpressed cyclins D1, E, and CDKs such as CDK2 and CDK4 drive uncontrolled growth. Agents such as polydatin induce a G2/M phase arrest, inhibiting the growth of HCC cells. These effects may be due to directly inhibiting CDK activity or increasing cell cycle inhibitors such as p21 and p27 to restore cell division control.⁷⁸⁻⁸⁰

7.4 Inhibition of Pro-Survival Pathways

Cancer cells often rely on constitutively active pro-survival signalling cascades to maintain their viability and growth under stress. Inhibition of these cascades is, therefore, a key therapeutic strategy, with several ameliorative agents in DEN-induced HCC models targeting these cascades. The Phosphoinositide 3-kinase (PI3K)/Akt signalling pathway is a key mediator of cell metabolism, growth, and survival, and is frequently hyperactivated in HCC.^{54,70,77-80} Activated Akt prevents cell death by suppressing pro-apoptotic proteins like Bad and caspase-9, activating anti-apoptotic proteins like Bcl-xL, and modulating downstream targets like mTOR, which is responsible for protein synthesis and cell growth. Some ameliorative agents have been shown to suppress the PI3K/Akt pathway in DEN-induced HCC models or HCC cell lines. For example, hesperidin, a flavonoid, possesses a protective role against chemically induced liver cancer by inhibiting the PI3K/Akt pathway. Likewise, polydatin has been found to decrease the levels of phosphorylated Akt.^{54,79} Through inhibition of this pathway, such agents reduce cell survival, decrease proliferation, and enhance apoptosis susceptibility, highlighting the need to target the PI3K/Akt/mTOR pathway in cancer treatment development.

Mitogen-Activated Protein Kinase (MAPK) signalling pathways—such as ERK, JNK, and p38 cascades—transmit extracellular signals to the nucleus, controlling processes such as proliferation, differentiation, stress responses, and apoptosis.^{69,79,90} Whereas ERK signalling is mostly associated with proliferation and survival, JNK and p38 pathways have the potential to trigger stress-associated apoptosis or inflammation based on cellular context. Dysregulation of these MAPK pathways occurs frequently in HCC.^{69,91} Ameliorative drugs regulate MAPK signalling to produce anti-cancer impacts by inhibiting pro-proliferative ERK signalling or stimulating pro-apoptotic JNK and p38 pathways. For instance, Notch1 activation in HCC cells regulates JNK signalling and enhances apoptosis. Polydatin also reduces phosphorylation of STAT3 and JAK1, which collaborate with MAPK pathways

downstream of receptor tyrosine kinases.^{79,90} By selective modulation of these branches of signalling, ameliorative agents tip the cellular balance in the direction of apoptosis and differentiation in opposition to tumour progression.

7.5 Epigenetic Regulation

Epigenetic alterations like DNA methylation, histone modification, and changes in non-coding RNA expression have important functions in HCC development by abnormally regulating tumorigenesis-related gene expression.⁷³ DEN-caused chronic liver damage induces generalised epigenetic changes that repress tumour suppressors or activate oncogenes. Certain ameliorative interventions have effects through the modulation of such epigenetic marks to re-establish normal patterns of gene expression. For instance, dietary restriction can modify the epigenetic landscape, possibly accounting for its protective effect against DEN-induced HCC.⁸⁶ While detailed epigenetic mechanisms are still to be understood for most agents, the Wnt/ β -catenin pathway is epigenetically controlled, with the restoration of Wnt antagonists such as SOX17, usually silenced by methylation, a potential therapeutic intervention.⁷³ Further, disruption of microRNAs governing major oncogenic pathways, such as mTOR or Wnt, is another epigenetic approach.⁴⁷ Additional studies are needed to better understand how certain agents alter DNA methylation, histone modification, and non-coding RNA expression in DEN-induced models.

7.6 Impact on the Tumour Microenvironment

The tumour microenvironment (TME) consists of cancer cells, stromal cells (fibroblasts and endothelial cells), immune cells (macrophages, lymphocytes, and neutrophils), and extracellular matrix components, all of which play an active role in tumour initiation, growth, invasion, and metastasis. DEN-induced chronic liver damage significantly remodels the TME by creating inflammation, angiogenesis, and immune suppression, thus enhancing tumour growth.^{67,69} Ameliorative interventions towards the TME attempt to suppress tumour growth by suppressing inflammation, inhibiting angiogenesis, or adjusting immune cell functions. Inhibition of NF- κ B activation and suppression of proinflammatory cytokines, as already outlined, have a direct impact on the inflammatory environment in the TME. Other compounds disrupt angiogenic signalling pathways like VEGF/VEGFR that are essential for tumour vascularisation.⁹² Curcumin has been investigated for anti-VEGF/VEGFR axis inhibitory activity, particularly in combination therapies. Modulating the metabolic phenotype of the TME or affecting immune cells such as tumour-associated macrophages (TAMs) and T lymphocytes could also augment anti-tumour responses.^{67,69,93,94} Through remodelling the supportive TME, these approaches make the environment less permissive to cancer cell survival and spread.

8. Challenges in Ameliorative Strategy Development

Developing potent ameliorative therapies for HCC is not easy despite encouraging preclinical outcomes

from models, including DEN-induced HCC. All these involve difficulties associated with efficacy, safety, delivery of the drug, and the ability to forecast patient reactions. Preclinical efficacy demonstrated in DEN models for clinical outcome is made difficult due to a variety of factors. Species variability in drug metabolism and bioavailability, the inherent heterogeneity of human HCC compared to animal models, and underlying liver cirrhosis in most patients all affect pharmacokinetics and drug tolerance.⁹⁵ Most drugs that show promise in preclinical models fail in clinical trials based on a lack of efficacy in humans or an unacceptable toxicity profile. Additionally, impaired liver function in HCC patients increases the risk of drug-induced liver injury and systemic side effects. Effective delivery of therapeutic agents to liver tumours also has additional challenges, particularly for systemically administered drugs. Nanotechnology-enabled delivery systems have the potential to enhance targeted drug deposition in tumour cells with less off-target toxicity; however, selective tumour targeting and minimising systemic exposure are ongoing research activities. Drug formulation optimisation and delivery to increase tumour uptake and decrease side effects is thus an important challenge.^{57,59,67,75,95}

A significant clinical hurdle is the unavailability of trustworthy biomarkers to identify patients who will respond to certain treatments. This leads to trial-and-error treatment strategies and restricts personalised therapy. DEN models are useful platforms for identifying prospective biomarkers linked to response or resistance to treatment by allowing extensive analysis of molecular alterations in tumours and nearby tissues after treatment. For instance, analysis of the expression of targeted pathways of signalling or oxidative stress and inflammation markers in treated animals can yield predictive signatures.^{79,95} Determination of such biomarkers in preclinical models can provide hypotheses that are further confirmed in clinical samples and may inform patient stratification. However, it is still challenging to find strong and easily quantifiable biomarkers that consistently translate from rodent models to patients.

9. Conclusion

Preclinical research based on the DEN-induced model of HCC has enormously contributed to an understanding of HCC progression and testing of candidate therapeutic approaches. Praised for its consistency and ability to simulate crucial phases of human HCC with concomitant chronic liver damage, the DEN model has played a dominant role in elucidating molecular events initiated by DEN treatment and probing ameliorative interventions. DEN triggers liver carcinogenesis through metabolic activation that leads to DNA damage and mutations, further fuelled by chronic inflammation and liver injury that promote survival and growth of mutated hepatocytes. At the core of DEN-induced HCC development are processes like DNA damage caused by reactive metabolites, interference with key signalling pathways like PI3K/Akt, MAPK, Wnt/ β -catenin, Hedgehog, and Notch, and the dual

functionality of ROS & RNS as both cellular damage and survival. Persistent inflammation induced by proinflammatory cytokines and NF- κ B activation provides a tumor-permissive microenvironment for cancer progression. Intra and inter-tumour heterogeneity are the major contributors to drug failure and recurrence of the disease, brought about by genetic, epigenetic, and microenvironmental heterogeneity between cancer cells. The development of high-throughput omics technologies—genomics, transcriptomics, proteomics, metabolomics, and epigenomics—provides unprecedented potential to uncover DEN-induced hepatocarcinogenesis and mechanisms of treatment.

Several promising ameliorative treatments are effective in preclinical models of DEN, from lifestyle and dietary regimens such as caloric restriction to natural products and nanoparticle-based agents. Nanotechnology holds promise for enhancing the delivery and efficacy of anti-HCC agents. Nanoparticles can encapsulate drugs to shield them from degradation, improve solubility, enable targeted delivery to tumour cells or liver tissue and reduce systemic toxicity. These strategies operate via multiple mechanisms, such as diminishing oxidative pressure by augmenting antioxidant protection, inhibiting inflammation through the prevention of cytokine secretion and NF- κ B signalling, re-establishing apoptosis and cell cycle control via caspase activation and modification of Bcl-2 and cyclins, preventing pro-survival signalling pathways like PI3K/Akt and MAPK, affecting epigenetic modifications, and enhancing tumour microenvironments. The DEN model is still a critical vehicle for studying HCC pathogenesis and response to treatment, successfully linking bench research with bedside application by facilitating the discovery of new targets and guiding trial design. Although it has limitations based on divergence from spontaneous human HCC and difficulty in extrapolation, its utility in preclinical HCC research is beyond dispute. In the future, studies based on DEN models will likely pay even greater attention to overcoming these weaknesses to bridge the gap between preclinical findings and improved prevention and therapy for patients with HCC.

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References:

- Nakayama J, Gong Z. Transgenic zebrafish for modeling hepatocellular carcinoma. *MedComm*. 2020;1(2):140-156. <https://doi.org/10.1002/mco.2.29> PMID:34766114 PMCID:PMC8491243
- Nahon P, Allaire M, Nault J-C, Paradis V. Characterizing the mechanism behind the progression of NAFLD to hepatocellular carcinoma. *Hepatic Oncol*. 2020;7(4). <https://doi.org/10.2217/hep-2020-0017> PMID:33680428 PMCID:PMC7907968
- Song X-J, Ma C-H. Mechanisms and immunotherapies of HBV- and NAFLD-related hepatocellular carcinoma. *Hepatoma Res*. 2020. <https://doi.org/10.20517/2394-5079.2020.05>
- Huang X, Wan F, Ma L, et al. Investigation of copper-cysteamine nanoparticles as a new photosensitizer for anti-hepatocellular carcinoma. *Cancer Biol Ther*. 2019;20(6):812-825. <https://doi.org/10.1080/15384047.2018.1564568> PMID:30727796 PMCID:PMC6606015
- Lencioni R, Braet F. Novel transarterial biomimetic-based nanoparticles for the treatment of hepatocellular carcinoma. *Gastroenterology*. 2016;150(2):312-314. <https://doi.org/10.1053/j.gastro.2015.12.022> PMID:26710988
- Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. *Eur J Pharmacol*. 2014;740:364-378. <https://doi.org/10.1016/j.ejphar.2014.07.025> PMID:25058905 PMCID:PMC4146684
- Matsuda Y, Wakai T, Kubota M, et al. Sorafenib-inhibited signaling: emerging evidence of RAF independent pathways as potential therapeutic targets in hepatocellular carcinoma. *InTech eBooks*. 2013. <https://doi.org/10.5772/55201>
- Shankaraiah RC, Gramantieri L, Fornari F, et al. Animal models of hepatocellular carcinoma prevention. *Cancers*. 2019;11(11):1792. <https://doi.org/10.3390/cancers11111792> PMID:31739536 PMCID:PMC6895981
- Yoon SK. Molecular mechanism of hepatocellular carcinoma. *Hepatoma Res*. 2018;4(8):42. <https://doi.org/10.20517/2394-5079.2018.23>
- Uehara T, Pogribny IP, Rusyn I. The DEN and CCl4-induced mouse model of fibrosis and inflammation-associated hepatocellular carcinoma. *Curr Protoc Pharmacol*. 2014;66(1). <https://doi.org/10.1002/0471141755.ph1430s66> PMID:25181010 PMCID:PMC4214366
- Merle P, Trepo C. Molecular mechanisms underlying hepatocellular carcinoma. *Viruses*. 2009;1(3):852-872. <https://doi.org/10.3390/v1030852> PMID:21994573 PMCID:PMC3185529
- Sarcognato S, García-Lezana T, Villanueva A. Mechanisms of action of drugs effective in hepatocellular carcinoma. *Clin Liver Dis*. 2019;14(2):62-65. <https://doi.org/10.1002/cld.810> PMID:31508222 PMCID:PMC6726381
- Rajesh Y, Sarkar D. Molecular mechanisms regulating obesity-associated hepatocellular carcinoma. *Cancers*. 2020;12(5):1290. <https://doi.org/10.3390/cancers12051290> PMID:32443737 PMCID:PMC7281233
- Zheng W, Deng M, Gong J. The molecular mechanism on the association of diabetes mellitus with hepatocellular carcinoma. *Int Surg J*. 2020;8(1):436. <https://doi.org/10.18203/2349-2902.isj20205921>
- Xiong XX, Qiu XY, Hu DX, Chen XQ. Advances in hypoxia-mediated mechanisms in hepatocellular carcinoma. *Mol Pharmacol*. 2017;92(3):246-255. <https://doi.org/10.1124/mol.116.107706> PMID:28242743

16. Zhao F, Korangy F, Greten TF. Cellular immune suppressor mechanisms in patients with hepatocellular carcinoma. *Dig Dis.* 2012;30(5):477-482. <https://doi.org/10.1159/000341695> PMID:23108303 PMCID:PMC3530892
17. Li E, Lin L, Chen C-W, Ou D-L. Mouse models for immunotherapy in hepatocellular carcinoma. *Cancers.* 2019;11(11):1800. <https://doi.org/10.3390/cancers11111800> PMID:31731753 PMCID:PMC6896030
18. Bagi CM, Andresen CJ. Models of hepatocellular carcinoma and biomarker strategy. *Cancers.* 2010;2(3):1441-1452. <https://doi.org/10.3390/cancers2031441> PMID:24281167 PMCID:PMC3837316
19. Ren Z, Chen X, Hong L, et al. Nanoparticle conjugation of ginsenoside Rg3 inhibits hepatocellular carcinoma development and metastasis. *Small.* 2019;16(2). <https://doi.org/10.1002/sml.201905233> PMID:31814271
20. Natri HM, Wilson MA, Buetow K. Distinct molecular etiologies of male and female hepatocellular carcinoma. Cold Spring Harbor Laboratory. 2018. <https://doi.org/10.1101/507939>
21. Luo Y, Song L, Wang X, et al. Uncovering the mechanisms of cryptotanshinone as a therapeutic agent against hepatocellular carcinoma. *Front Pharmacol.* 2020;11. <https://doi.org/10.3389/fphar.2020.01264> PMID:32903546 PMCID:PMC7438559
22. Qin J, Yang L, Sheng X, et al. Antitumor effects of brucine immunonanoparticles on hepatocellular carcinoma in vivo. *Oncol Lett.* 2018. <https://doi.org/10.3892/ol.2018.8168>
23. Schulien I, Hasselblatt P. Diethylnitrosamine-induced liver tumorigenesis in mice. *Methods Cell Biol.* 2020:137-152. <https://doi.org/10.1016/bs.mcb.2020.08.006> PMID:33785162
24. Singh D, Singh M, Yadav E, et al. Attenuation of diethylnitrosamine (DEN) - induced hepatic cancer in experimental model of Wistar rats by Carissa carandas embedded silver nanoparticles. *Biomed Pharmacother.* 2018;108:757-765. <https://doi.org/10.1016/j.biopha.2018.09.066> PMID:30248544
25. Singh D, Singh M, Yadav E, et al. Amelioration of diethylnitrosamine (DEN)-induced hepatocellular carcinogenesis in animal models via knockdown of oxidative stress and proinflammatory markers by Madhuca longifolia embedded silver nanoparticles. *RSC Adv.* 2018;8(13):6940-6953. <https://doi.org/10.1039/C7RA12775H> PMID:35540326 PMCID:PMC9078335
26. Singh R, Sharma J, Goyal PK. Prophylactic role of Averrhoa carambola (star fruit) extract against chemically induced hepatocellular carcinoma in swiss albino mice. *Adv Pharmacol Sci.* 2014;1-8. <https://doi.org/10.1155/2014/158936> PMID:24696677 PMCID:PMC3950398
27. Thamer NA. Green synthesized silver nanoparticles using Crocus sativus L extract after reduces prehepatocellular carcinoma in rats. *Iraqi J Cancer Med Genet.* 2018;9(2). <https://doi.org/10.29409/ijcmg.v9i2.191>
28. Andersen JB, Loi R, Perra A, et al. Progenitor-derived hepatocellular carcinoma model in the rat. *Hepatology.* 2010;51(4):1401-1409. <https://doi.org/10.1002/hep.23488> PMID:20054870 PMCID:PMC2914300
29. Shirato K, Morimoto M, Tomita N, et al. Hepatocellular carcinoma. *J Ultrasound Med.* 2002;21(9):1015-1022. <https://doi.org/10.7863/jum.2002.21.9.1015> PMID:12216749
30. Bu L, Xie J, Chen K, et al. Assessment and comparison of magnetic nanoparticles as MRI contrast agents in a rodent model of human hepatocellular carcinoma. *Contrast Media Mol Imaging.* 2012;7(4):363-372. <https://doi.org/10.1002/cmmi.494> PMID:22649042 PMCID:PMC3759163
31. Hu P, Fu Z, Liu G, et al. Gadolinium-based nanoparticles for theranostic MRI-guided radiosensitization in hepatocellular carcinoma. *Front Bioeng Biotechnol.* 2019;7. <https://doi.org/10.3389/fbioe.2019.00368> PMID:31828068 PMCID:PMC6890599
32. You Y, Zhu F, Li Z, et al. Phyllanthin prevents diethylnitrosamine (DEN) induced liver carcinogenesis in rats and induces apoptotic cell death in HepG2 cells. *Biomed Pharmacother.* 2021;137:111335. <https://doi.org/10.1016/j.biopha.2021.111335> PMID:33581648
33. Liu L, Wang B, Ma Y, et al. A review of Phyllanthus urinaria L. in the treatment of liver disease: viral hepatitis, liver fibrosis/cirrhosis and hepatocellular carcinoma. *Front Pharmacol.* 2024;15. <https://doi.org/10.3389/fphar.2024.1443667> PMID:39185304 PMCID:PMC11341462
34. Wang J, Li C-D, Sun L. Recent advances in molecular mechanisms of the NKG2D pathway in hepatocellular carcinoma. *Biomolecules.* 2020;10(2):301. <https://doi.org/10.3390/biom10020301> PMID:32075046 PMCID:PMC7094213
35. Liu Y, Yang Z, Du F, et al. Molecular mechanisms of pathogenesis in hepatocellular carcinoma revealed by RNA-sequencing. *Mol Med Rep.* 2017;16(5):6674-6682. <https://doi.org/10.3892/mmr.2017.7457> PMID:28901494 PMCID:PMC5865798
36. Li Y, Hecht SS. Metabolic activation and DNA interactions of carcinogenic N-nitrosamines to which humans are commonly exposed. *Int J Mol Sci.* 2022;23(9):4559. <https://doi.org/10.3390/ijms23094559> PMID:35562949 PMCID:PMC9105260
37. Boysen G, Pachkowski BF, Nakamura J, Swenberg JA. The formation and biological significance of N7-guanine adducts. *Mutat Res Genet Toxicol Environ Mutagen.* 2009;678(2):76-94. <https://doi.org/10.1016/j.mrgentox.2009.05.006> PMID:19465146 PMCID:PMC2739241
38. Perrino FW, Blans P, Harvey S, et al. The N2-ethylguanine and the O6-ethyl- and O6-methylguanine lesions in DNA: contrasting responses from the "bypass" DNA polymerase η and the replicative DNA polymerase α . *Chem Res Toxicol.* 2003;16(12):1616-1623. <https://doi.org/10.1021/tx034164f> PMID:14680376
39. Nakamura YK, Omaye ST. Age-related changes of serum lipoprotein oxidation in rats. *Life Sci.* 2003;74(10):1265-1275. <https://doi.org/10.1016/j.lfs.2003.08.007> PMID:14697409
40. Kang JS, Wanibuchi H, Morimura K, et al. Role of CYP2E1 in diethylnitrosamine-induced hepatocarcinogenesis in vivo. *Cancer Res.* 2007;67(23):11141-11146. <https://doi.org/10.1158/0008-5472.CCR-07-1369> PMID:18056438
41. Arboatti AS, Lambertucci F, Sedlmeier MG, et al. Diethylnitrosamine increases proliferation in early stages of hepatic carcinogenesis in insulin-treated type 1 diabetic mice. *BioMed Res Int.* 2018;1-10. <https://doi.org/10.1155/2018/9472939> PMID:29850590 PMCID:PMC5937583
42. Takaki A. Control of oxidative stress in hepatocellular carcinoma: helpful or harmful? *World J Hepatol.* 2015;7(7):968. <https://doi.org/10.4254/wjh.v7.i7.968> PMID:25954479 PMCID:PMC4419100
43. Zhang L, Chen C, Duanmu J, et al. Cryptotanshinone inhibits the growth and invasion of colon cancer by suppressing inflammation and tumor angiogenesis through modulating MMP/TIMP system, PI3K/Akt/mTOR signaling and HIF-1 α nuclear translocation. *Int Immunopharmacol.* 2018;65:429-437. <https://doi.org/10.1016/j.intimp.2018.10.035> PMID:30388517
44. Cordover E, Minden A. Signaling pathways downstream to receptor tyrosine kinases: targets for cancer treatment. *J Cancer Metastasis Treat.* 2020. <https://doi.org/10.20517/2394-4722.2020.101>
45. Martinez-Quetglas I, Pinyol R, Dauch D, et al. IGF2 is up-regulated by epigenetic mechanisms in hepatocellular carcinomas and is an actionable oncogene product in experimental models. *Gastroenterology.* 2016;151(6):1192-1205. <https://doi.org/10.1053/j.gastro.2016.09.001> PMID:27614046
46. Aravalli RN, Steer CJ, Cressman ENK. Molecular mechanisms of hepatocellular carcinoma. *Hepatology.* 2008;48(6):2047-2063. <https://doi.org/10.1002/hep.22580> PMID:19003900

47. Liang Y, Liang Q, Qiao L, Xiao F. MicroRNAs modulate drug resistance-related mechanisms in hepatocellular carcinoma. *Front Oncol.* 2020;10. <https://doi.org/10.3389/fonc.2020.00920> PMID:32695666 PMCID:PMC7338562
48. Qi S-X, Sun H, Liu H, et al. Role and mechanism of circ-PRKCI in hepatocellular carcinoma. *World J Gastroenterol.* 2019;25(16):1964-1974. <https://doi.org/10.3748/wjg.v25.i16.1964> PMID:31086464 PMCID:PMC6487381
49. SRIGOPALRAM S, JAYRAAJ IA. EFFECT OF TERMINALIA CHEBULA RETZ ON DEN INDUCED HEPATOCELLULAR CARCINOGENESIS IN EXPERIMENTAL RATS. *Int J Pharm Pharm Sci.* 2012;4(2):440-445. <https://doi.org/10.1016/j.bionut.2011.12.003>
50. Krishnan GS, Rajagopal V, Joseph SRA, et al. In vitro, in silico and in vivo antitumor activity of crude methanolic extract of Tetilla dactyloidea (carter, 1869) on DEN induced HCC in a rat model. *Biomed Pharmacother.* 2017;95:795-807. <https://doi.org/10.1016/j.biopha.2017.08.054> PMID:28892791
51. Vidya R, Kalaivani K, Amudha P. Therapeutic potential of Cucumis melo (L.) fruit extract and its silver nanoparticles against DEN-induced hepatocellular cancer in rats. *Appl Biochem Biotechnol.* 2021;194(1):368-381. <https://doi.org/10.1007/s12010-021-03765-9> PMID:34792748
52. Cheng M, He B, Wan T, et al. 5-fluorouracil nanoparticles inhibit hepatocellular carcinoma via activation of the p53 pathway in the orthotopic transplant mouse model. *PLoS One.* 2012;7(10):e47115. <https://doi.org/10.1371/journal.pone.0047115> PMID:23077553 PMCID:PMC3471936
53. Hassan SK, Mousa AM, Eshak MG, et al. Therapeutic and chemopreventive effects of nano curcumin against diethylnitrosamine induced hepatocellular carcinoma in rats. *Int J Pharm Pharm Sci.* 2014;6(3):54-62. <http://www.ijppsjournal.com/Vol6Issue3/8477.pdf>
54. Mo'men YS, Hussein RM, Kandeil MA. Involvement of PI3K/Akt pathway in the protective effect of hesperidin against a chemically induced liver cancer in rats. *J Biochem Mol Toxicol.* 2019;33(6). <https://doi.org/10.1002/jbt.22305M> PMID:30779474
55. Sarkar S, Das AK, Bhattacharya S, et al. Isorhamnetin exerts anti-tumor activity in DEN + CCl4-induced HCC mice. *Med Oncol.* 2023;40(7). <https://doi.org/10.1007/s12032-023-02050-5> PMID:37226027
56. Chenthamara D, Subramaniam S, Ramakrishnan SG, et al. Therapeutic efficacy of nanoparticles and routes of administration. *Biomater Res.* 2019;23(1). <https://doi.org/10.1186/s40824-019-0166-x> PMID:31832232 PMCID:PMC6869321
57. Metkar SP, Fernandes G, Navti PD, et al. Nanoparticle drug delivery systems in hepatocellular carcinoma: a focus on targeting strategies and therapeutic applications. *OpenNano.* 2023;12:100159. <https://doi.org/10.1016/j.onano.2023.100159>
58. Elaidy SM, Moghazy A, El-Kherbetawy MK. Evaluation of the therapeutic effects of Polyvinylpyrrolidone-Capped silver nanoparticles on the Diethylnitrosamine/Carbon Tetrachloride-Induced hepatocellular carcinoma in rats. *Egypt J Basic Clin Pharmacol.* 2017;7. <https://doi.org/10.32527/2017/101422>
59. Chugh H, Sood D, Chandra I, et al. Role of gold and silver nanoparticles in cancer nano-medicine. *Artif Cells Nanomed Biotechnol.* 2018;46(sup1):1210-1220. <https://doi.org/10.1080/21691401.2018.1449118> PMID:29533101
60. Khaled AM, Othman MS, Obeidat ST, et al. Green-synthesized silver and selenium nanoparticles using berberine: a comparative assessment of in vitro anticancer potential on human hepatocellular carcinoma cell line (HepG2). *Cells.* 2024;13(3):287. <https://doi.org/10.3390/cells13030287> PMID:38334679 PMCID:PMC10854975
61. Mohamed MR, Osman SA, Hassan AA, et al. Gemcitabine and synthesized silver nanoparticles impact on chemically induced hepatocellular carcinoma in male rats. *Int J Immunopathol Pharmacol.* 2024;38. <https://doi.org/10.1177/03946320241263352> PMID:39046434 PMCID:PMC11271163
62. Uchida D, Takaki A, Oyama A, et al. Oxidative stress management in chronic liver diseases and hepatocellular carcinoma. *Nutrients.* 2020;12(6):1576. <https://doi.org/10.3390/nu12061576> PMID:32481552 PMCID:PMC7352310
63. Kouroumalis E, Tsomidis I, Voumvouraki A. Pathogenesis of hepatocellular carcinoma: the interplay of apoptosis and autophagy. *Biomedicines.* 2023;11(4):1166. <https://doi.org/10.3390/biomedicines11041166> PMID:37189787 PMCID:PMC10135776
64. Elmetwalli A, Abdel-Monem MO, El-Far AH, et al. Probiotic-derived silver nanoparticles target mTOR/MMP-9/BCL-2/dependent AMPK activation for hepatic cancer treatment. *Med Oncol.* 2024;41(5). <https://doi.org/10.1007/s12032-024-02330-8> PMID:38575697 PMCID:PMC10995097
65. Ahmadian E, Dizaj SM, Rahimpour E, et al. Effect of silver nanoparticles in the induction of apoptosis on human hepatocellular carcinoma (HepG2) cell line. *Mater Sci Eng C.* 2018;93:465-471. <https://doi.org/10.1016/j.msec.2018.08.027> PMID:30274079
66. Tanaka S, Mogushi K, Yasen M, et al. Oxidative stress pathways in noncancerous human liver tissue to predict hepatocellular carcinoma recurrence: a prospective, multicenter study. *Hepatology.* 2011;54(4):1273-1281. <https://doi.org/10.1002/hep.24536> PMID:22006857
67. Yang JD, Nakamura I, Roberts LR. The tumor microenvironment in hepatocellular carcinoma: current status and therapeutic targets. *Semin Cancer Biol.* 2010;21(1):35-43. <https://doi.org/10.1016/j.semcancer.2010.10.007> PMID:20946957 PMCID:PMC3050428
68. Moeini A, Cornella H, Villanueva A. Emerging signaling pathways in hepatocellular carcinoma. *Liver Cancer.* 2012;1(2):83-93. <https://doi.org/10.1159/000342405> PMID:24159576 PMCID:PMC3747540
69. Huang P, Xu X, Wang L, et al. The role of EGF-EGFR signalling pathway in hepatocellular carcinoma inflammatory microenvironment. *J Cell Mol Med.* 2013;18(2):218-230. <https://doi.org/10.1111/jcmm.12153> PMID:24268047 PMCID:PMC3930409
70. Huang Q, Li J, Zheng J, Wei A. The carcinogenic role of the Notch signaling pathway in the development of hepatocellular carcinoma. *J Cancer.* 2019;10(6):1570-1579. <https://doi.org/10.7150/jca.26847> PMID:31031867 PMCID:PMC6485212
71. Ferrín G, Guerrero M, Amado V, et al. Activation of mTOR signaling pathway in hepatocellular carcinoma. *Int J Mol Sci.* 2020;21(4):1266. <https://doi.org/10.3390/ijms21041266> PMID:32070029 PMCID:PMC7072933
72. Jeng K, Jeng C, Jeng W, et al. Sonic hedgehog signaling pathway as a potential target to inhibit the progression of hepatocellular carcinoma (review). *Oncol Lett.* 2019. <https://doi.org/10.3892/ol.2019.10826> PMID:31611946 PMCID:PMC6781692
73. Jia Y, Yang Y, Liu S, et al. SOX17 antagonizes WNT/ β -catenin signaling pathway in hepatocellular carcinoma. *Epigenetics.* 2010;5(8):743-749. <https://doi.org/10.4161/epi.5.8.13104> PMID:20716954
74. Wang X, Xiang L, Li H, et al. The role of HMGB1 signaling pathway in the development and progression of hepatocellular carcinoma: a review. *Int J Mol Sci.* 2015;16(9):22527-22540. <https://doi.org/10.3390/ijms160922527> PMID:26393575 PMCID:PMC4613322
75. Kudo M. Signaling pathway and molecular-targeted therapy for hepatocellular carcinoma. *Dig Dis.* 2011;29(3):289-302. <https://doi.org/10.1159/000327562> PMID:21829020
76. YANG Z, YANG S, MISNER BJ, LIU-SMITH F, MEYSKENS FL. The role of APE/ref-1 signaling pathway in hepatocellular carcinoma

- progression. *Int J Oncol.* 2014;45(5):1820-1828. <https://doi.org/10.3892/ijo.2014.2589> PMID:25109342 PMCID:PMC4735689
77. Huang X-Y, Huang Z-L, Zhang P-B, et al. CircRNA-100338 is associated with mTOR signaling pathway and poor prognosis in hepatocellular carcinoma. *Front Oncol.* 2019;9. <https://doi.org/10.3389/fonc.2019.00392> PMID:31157168 PMCID:PMC6528706
78. Wang X, Zhang J, Zhou L, et al. Fbxw7 regulates hepatocellular carcinoma migration and invasion via Notch1 signaling pathway. *Int J Oncol.* 2015;47(1):231-243. <https://doi.org/10.3892/ijo.2015.2981> PMID:25955618
79. Jiang J, Chen Y, Dong T, et al. Polydatin inhibits hepatocellular carcinoma via the AKT/STAT3 FOXO1 signaling pathway. *Oncol Lett.* 2019. <https://doi.org/10.3892/ol.2019.10123>
80. Li Y, Li P, Wang N. Effect of let 7c on the PI3K/Akt/FoxO signaling pathway in hepatocellular carcinoma. *Oncol Lett.* 2020;21(2). <https://doi.org/10.3892/ol.2020.12357> PMID:33376529 PMCID:PMC7751369
81. Wang J, Tian Y, Chen H, et al. Key signaling pathways, genes and transcription factors associated with hepatocellular carcinoma. *Mol Med Rep.* 2018. <https://doi.org/10.3892/mmr.2018.8871>
82. Nie X, Liu Y, Chen W-D, Wang Y-D. Interplay of miRNAs and canonical Wnt signaling pathway in hepatocellular carcinoma. *Front Pharmacol.* 2018;9. <https://doi.org/10.3389/fphar.2018.00657> PMID:29977206 PMCID:PMC6021530
83. Ciccarone F, Castelli S, Ciriolo MR. Oxidative stress-driven autophagy acROs onset and therapeutic outcome in hepatocellular carcinoma. *Oxid Med Cell Longev.* 2019;1-10. <https://doi.org/10.1155/2019/6050123> PMID:31205585 PMCID:PMC6530208
84. Shimizu Y, Tamura T, Kemmochi A, et al. Oxidative stress and liver X receptor agonist induce hepatocellular carcinoma in non-alcoholic steatohepatitis model. *J Gastroenterol Hepatol.* 2020;36(3):800-810. <https://doi.org/10.1111/jgh.15239> PMID:32870526 PMCID:PMC7983938
85. McLoughlin MR, Orlicky DJ, Prigge JR, et al. TrxR1, Gsr, and oxidative stress determine hepatocellular carcinoma malignancy. *Proc Natl Acad Sci USA.* 2019;116(23):11408-11417. <https://doi.org/10.1073/pnas.1903244116> PMID:31097586 PMCID:PMC6561278
86. Duan T, Sun W, Zhang M, et al. Dietary restriction protects against diethylnitrosamine-induced hepatocellular tumorigenesis by restoring the disturbed gene expression profile. *Sci Rep.* 2017;7(1). <https://doi.org/10.1038/srep43745> PMID:28262799 PMCID:PMC5338348
87. Hashem MA, Zidan AZM, El-Mandrawy SA. POTENTIAL PERFECTION EFFECTS OF SILVER NANOPARTICLES AGAINST CISPLATIN SIDE EFFECTS IN HEPATOCELLULAR CARCINOMA INDUCED IN SPRAGUE DAWLEY ALBINO RATS: HEMATOLOGICAL, BIOCHEMICAL, HISTOPATHOLOGICAL, AND IMMUNOHISTOCHEMICAL ALTERATIONS. *Sloven Vet Res.* 2023;60(25-Suppl). <https://doi.org/10.26873/SVR-1554-2022>
88. Petri S, Körner S, Kiaei M. Nrf2/ARE signaling pathway: key mediator in oxidative stress and potential therapeutic target in ALS. *Neurol Res Int.* 2012;1-7. <https://doi.org/10.1155/2012/878030> PMID:23050144 PMCID:PMC3461296
89. Prasannaraj G, Venkatachalam P. Hepatoprotective effect of engineered silver nanoparticles coated bioactive compounds against diethylnitrosamine induced hepatocarcinogenesis in experimental mice. *J Photochem Photobiol B.* 2017;167:309-320. <https://doi.org/10.1016/j.jphotobiol.2017.01.009> PMID:28129629
90. Sui C, Zhuang C, Sun D, et al. Notch1 regulates the JNK signaling pathway and increases apoptosis in hepatocellular carcinoma. *Oncotarget.* 2017;8(28):45837-45847. <https://doi.org/10.18632/oncotarget.17434> PMID:28507277 PMCID:PMC5542231
91. HAYAKAWA R, HAYAKAWA T, TAKEDA K, ICHIJO H. Therapeutic targets in the ASK1-dependent stress signaling pathways. *Proc Jpn Acad Ser B Phys Biol Sci.* 2012;88(8):434-453. <https://doi.org/10.2183/pjab.88.434> PMID:23060232 PMCID:PMC3491083
92. Wu X, Xie G, Chen D. Hypoxia and hepatocellular carcinoma: the therapeutic target for hepatocellular carcinoma. *J Gastroenterol Hepatol.* 2007;22(8):1178-1182. <https://doi.org/10.1111/j.1440-1746.2007.04997.x> PMID:17559361
93. Mossenta M, Busato D, Dal Bo M, Toffoli G. Glucose metabolism and oxidative stress in hepatocellular carcinoma: role and possible implications in novel therapeutic strategies. *Cancers.* 2020;12(6):1668. <https://doi.org/10.3390/cancers12061668> PMID:32585931 PMCID:PMC7352479
94. GAO J-Z, DU J-L, WANG Y-L, et al. Synergistic effects of curcumin and bevacizumab on cell signaling pathways in hepatocellular carcinoma. *Oncol Lett.* 2014;9(1):295-299. <https://doi.org/10.3892/ol.2014.2694> PMID:25435978 PMCID:PMC4246621
95. Gong J, Chuang J, Cho M, et al. Molecular targets, pathways, and therapeutic implications for hepatocellular carcinoma. *Int J Mol Sci.* 2020;21(15):5232. <https://doi.org/10.3390/ijms21155232> PMID:32718047 PMCID:PMC7432744