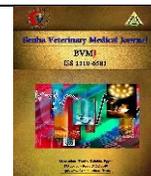




Official Journal Issued by
Faculty of
Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: <https://bvmj.journals.ekb.eg/>



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Original Paper

Chemotherapeutic and antiangiogenic activity of Ginger and Ginger nanoparticles in hepatocarcinogenesis -induced in rats via activation of miRNA-29 and attenuation of FGF2/HGF/ TGF- β 1 signaling pathways

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ARTICLE INFO

Keywords

Hepatocellular carcinoma,
Ginger extract,
Ginger nanoparticles,
Angiogenesis,
Epigenetic
miRNA-29

Received 21/01/2024

Accepted 14/02/2024

Available On-Line

01/04/2024

ABSTRACT

The antioxidative, anti-inflammatory, anticancer, and hepatoprotective qualities of ginger have been well-established. This research was purposed to evaluate both ginger extract (GE) and ginger nanoparticles (GNPs) potential chemotherapeutic influences in mitigating hepatocellular carcinoma (HCC) induced by carbon tetrachloride (CCl₄) and diethylnitrosamine (DEN) in rats. HCC was induced in rats through DEN injection (200 mg/kg b.w./I.P.). Subsequently, after a two-week interval, rats were exposed to three consecutive weekly doses of CCL4 (3 mL/kg b.w. orally), prepared in a 1:1 dilution with corn oil, serving as a carcinogenic promoter. CCL4 and DEN administration were repeated following an additional 5-week interval. Fifteen weeks after HCC induction, oral treatment with GE (300 mg/kg b.w./day) and GNPs (50 mg/kg b.w./day) was initiated and continued for 6 weeks. Twenty-eight rats were divided evenly into four subgroups: G1 (normal control), G2 (DEN/CCL4 induced HCC), G3 (DEN/CCL4+GE), and G4 (DEN/CCL4+GNPs). The results demonstrated a substantial elevation in AST, ALT, and ALP serum activities and a decrease in liver microRNA-29 expression in rats with induced hepatocellular carcinoma. High levels of TGF- β 1, FGF, and HGF also pointed to upregulation during the development of HCC. Treatment with GE and GNPs led to a significant reduction in liver marker enzymes and the heightened expression of TGF- β 1, FGF2, and HGF, accompanied by an upregulation of microRNA-29 in rats with liver cancer. In conclusion, GE and GNP treatments may serve as chemotherapeutic agents by activating the tumor suppressor microRNA-29 gene and suppressing angiogenesis growth factors in the liver.

1. INTRODUCTION

Liver cancer remains one of the most widespread malignancies worldwide and stands as the second leading cause of death (Zhou et al., 2016). Chemoprotection strategies are often needed to effectively deal with the effects of cancer. These involve using natural foods and outside phytochemicals to boost the body's natural defenses against different stages of cancer growth (Dimarco-Crook et al., 2015). The primary risk factors linked to hepatocellular carcinoma encompass viral infections like hepatitis B and C, exposure to environmental carcinogens, alcohol misuse, and fatty liver disease (Zhou et al., 2016). Diethylnitrosamine (DEN) is a common human carcinogen found in various sources, including cigarettes, cosmetics, gasoline, and certain food items like meat and milk (Santos et al., 2017). Employed as a widely used carcinogenic agent in experimental animal

models, DEN induces the development of diverse tumors, affecting organs such as the liver, gastrointestinal tract, skin, respiratory tract, and hematopoietic cells when administered orally or intraperitoneally to mice. Numerous researchers have utilized DEN to induce liver tumors in mice, administering it through intraperitoneal injection two weeks after birth in weaning mice, leading to the emergence of hepatic tumors approximately eight months later (Fan et al., 2010). As DEN lacks intrinsic carcinogenicity, it requires bio-activation by cytochrome P450 (CYP) enzymes in the liver, leading to the formation of DNA adducts through an alkylation mechanism (Verna et al., 1996).

The alkylation adducts formed can be efficiently repaired by the DNA repair gene 06-methylguanine-DNA methyltransferase (MGMT), also known as 06-alkylguanine-DNA methyltransferase (Jacinto and Esteller, 2007). Earlier investigations conducted by

Kang et al. (2007) revealed that mice lacking the cytochrome P450 enzyme 2E1 (CYP2E1) demonstrated tumors multiplicity and decreased occurrence compared to wild-type (WT) mice in the context of DEN-induced hepatocarcinogenesis. This observation strongly indicates the crucial role of CYP2E1 in the activation of DEN, despite the consideration of various other cytochrome P450 enzymes for catalyzing DEN bioactivation in vivo (Verna et al., 1996).

Carbon tetrachloride is widely employed as an industrial chemical due to its propensity for easy evaporation into the surrounding environment. The liver, housing the cytochrome P450 enzyme system, demonstrates a notable affinity for CCl₄, facilitating its transformation into trichloromethyl radical (CCl₃) and peroxy trichloromethyl radical (CCl₃O₂) radicals. The excessive production of these noxious metabolites can covalently attach to proteins and lipids in the plasma membrane or interact with oxygen, initiating lipid peroxidation. Additionally, the heightened generation of free radicals may result in the depletion or inactivation of antioxidant enzymes. These effects contribute to the impairment of unsaturated fatty acids, giving rise to a range of pathological issues (Ghadi et al., 2019).

Zingiber Officinale Roscoe's rhizome, within the Zingiber family, is considered the origin of ginger and holds a versatile role as a flavoring agent, spice, and supplement. Its historical use extends to treating various ailments, for instance, arthritis, colds, rheumatism, and nausea (Ali et al., 2008). Notably, ginger possesses a diverse range of medicinal properties encompassing antibacterial, inflammation-reducing effects, anti-insecticidal, antiviral, antiparasitic, antifungal, analgesic, antimutagenic, anticarcinogenic and antioxidant properties (Shanmugam et al., 2021).

When combined with natural product interventions and chemotherapy, nanoparticle (NP)-based targeted drug delivery has even more benefits (Watkins et al., 2015). Formulations that integrate NPs with chemotherapeutic agents and natural products exhibit an increased therapeutic index, facilitating improved delivery to tumor tissues. This improvement is attributed to enhanced retention, permeability, favourable pharmacokinetic profiles, and a reduction in adverse events. NPs play a crucial role in reducing permeability across healthy capillaries, thereby mitigating toxicity and bioavailability in normal tissues (Gaster et al., 2015).

Chitosan, derived from chitin, is a progressively utilized component in tissue engineering and drug delivery. Since chitosan is cheap, biocompatible, biodegradable, and doesn't cause much harm, it is widely used as an NP formulation medium in wound healing products and controlled-release dosage forms (Quinones et al., 2018). Consequently, the potential chemotherapeutic effect of ginger and ginger nanoparticles on alterations of certain epigenetic and molecular markers, in addition to liver tissue

histopathological examination, was assessed in an experimental model of HCC in rats.

2. MATERIALS AND METHODS

2.1. Experimental animals

Thirty-two male albino rats, aged between 4 and 5 weeks, with a weight ranging from 100 to 150 g, were purchased from the laboratory animal research center, faculty of Veterinary Medicine, Benha University. Rats are housed in separate wire mesh cages, exposed to good ventilation, humidity, and a 12-hour light-dark cycle. Animals are provided with a constant supply of a standard pellet diet and fresh, clean drinking water ad libitum. Rats are left for 15 days before the beginning of the experiment for adaptation. The experimental protocol was conducted according to the guide for the Institutional Animal Care and Use Committee approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Benha University (BUFVTM 14-03-23).

2.2. Chemicals and natural agents

2.2.1. Diethylnitrosoamine (DEN), also referred to as (N-Nitrosodiethylamine)

The transparent yellow liquid form of N-nitroso diethylamine (1 g/1 ml vial) was acquired from Sigma Aldrich Company for Trading Chemicals, Medicines, and Medical Appliances in Egypt. The substance was recently prepared in normal saline and given to the rats through intraperitoneal injection at a dosage of 200 mg/kg body weight (Singh et al., 2009).

2.2.2. Carbon Tetrachloride (CCl₄):

The colorless liquid carbon tetrachloride was purchased from the El-Gomhouria Company for Trading Chemicals and Medical Appliances in Egypt. CCl₄ was freshly prepared in corn oil at a (1:1) dilution, followed by oral administration of 3 mL/kg b.wt. to the rats as a promoter of carcinogenesis (Hassan et al., 2014).

2.2.3. Ginger extract (GE)

Ginger extract was purchased from Nano Gate Company, Egypt, 11571 (for Scientific Services).

Preparation of ginger extract:

The ginger rhizomes underwent drying in an oven at a temperature of 50–60 °C for 24 hours. Subsequently, the dried ginger was ground using a mechanical mixer, and the ginger powder was extracted through maceration using distilled water as a solvent (maintaining a powder-to-water ratio of 1:10). The extraction process was carried out at a temperature of 75 °C for 24 hours. Additionally, filtration was conducted using filter paper to separate the extract or filtrate from the solid waste. To store the ginger extracts, dark amber bottles were utilized, maintaining a temperature of 8 °C (Manuhara et al., 2018).

Ginger extract was orally administered (300 mg/kg b. wt./day) for six weeks (Baker et al., 2019).

2.2.4. Ginger nanoparticles (GNP)

Purchased from Nano Gate Company, 11571, Egypt (for scientific services).

Preparation Method:

a-Chitosan nanoparticles:

The preparation of chitosan nanoparticles followed the ionotropic gelation process as outlined by Hasanin et al. (2018). Blank nanoparticles were created by introducing a tripolyphosphate (TPP) aqueous solution into a chitosan solution. Briefly, a solution of uniform consistency was achieved by dissolving 1 g of chitosan powder in 200 ml of 1% acetic acid (pH = 4). The mixture was then agitated for 6 hours. Following this, 150 ml of TPP 0.2% w/v was added drop by drop. The initially clear solution underwent a transition to turbidity, indicating the formation of chitosan nanoparticles (CSNPs). Subsequently, the resulting suspension underwent three washes through centrifugation for 30 minutes at 12,000 rpm (Hermle Z32 HK, Germany) with deionized water (DH2O).

b. preparation of ginger-encapsulated chitosan nanoparticles (GNPs)

The ginger extract, previously prepared, was incorporated into the chitosan solution with slight adjustments following the method described above. In brief, 1g of ginger extract was dissolved in the chitosan solution at a weight ratio of 10%. After 20 minutes of magnetic stirring, tripolyphosphate was slowly added to the solution. Centrifugation at 4 °C and 12,000g for 30 minutes was used to separate the chitosan nanoparticles. The ginger nanoparticles (GNPs) were taken orally once daily for six weeks at a dose of 50 mg/kg body weight (Elrahman et al., 2020).

Induction of hepatocarcinogenesis:

Diethylnitrosamine (DEN) in normal saline was administered intraperitoneally to rats at a dose of 200 mg/kg body weight to induce hepatocellular carcinoma, as described by Singh et al. (2009). Subsequently, two weeks after the initial DEN injection, rats were administered three successive weekly doses of carbon tetrachloride (CCl₄) diluted in corn oil at a 1:1 ratio and given orally at 3 ml/kg body weight as a promoter of carcinogenic effect. The administration of DEN and CCl₄ was repeated once again after 5 weeks from the onset of the DEN injection (Hassan et al., 2014). Fifteen weeks after the induction of HCC, treatment with ginger extract and ginger nanoparticles was initiated and continued for six weeks.

2.3. Experimental design

The rats were allocated into four equal groups, with each group consisting of 8 rats:

Group 1 (normal control): The rats were given a standard diet and injected with saline IP for the whole 21 weeks (the experimental period).

Group 2 (DEN/CCl₄): a dosage of 200 mg/kg body weight of DEN in normal saline was administered intraperitoneally to rats to develop hepatocellular carcinoma (HCC). Then, after fourteen days, rats were orally given a dose of carbon tetrachloride (CCl₄) diluted in corn oil at a 1:1 ratio (3 ml/kg body weight). This acted as a promoter to enhance the carcinogenic impact. After 5 weeks had passed since the first DEN injection, the rats were given another injection of both DEN and CCl₄.

Group 3 (DEN/ CCl₄ + GE): Rats in this group received DEN and CCl₄, following the protocol outlined in Group 2. Subsequently, they underwent post-treatment with ginger extract (GE) at an oral dosage of (300 mg/kg b.wt./day). This post-treatment started 15 weeks after the administration of DEN and CCl₄ and continued for 6 weeks.

Group 4 (DEN/CCl₄ + GNPs): Rats received DEN and CCl₄ injections as in Group 2 and were post-treated with GNPs (50 mg/kg b.wt./day) orally after 15 weeks from the administration of DEN and CCl₄ for 6 weeks. Throughout the experimental period, the dosage was regularly adjusted every week, following any variations in body weight. This adjustment was undertaken to ensure a consistent dose per kg of body weight for each group throughout the entire study duration.

2.4. Sampling:

Blood samples:

Blood samples were taken at random by ocular vein puncture and stored in screw-capped tubes. Centrifugation was used for 15 minutes at 3000 r.p.m. to separate the serum. The serum was transferred to dry sterile sample tubes using automated pipettes and then stored in a deep freeze at -20 °C until use for the determination of liver marker enzymes.

Tissue specimens

Following the guidelines set forth by the Animal Ethics Committees, the rats' euthanasia took place after the 21-week experiment. The abdomen was then opened, and the liver was carefully removed for subsequent analysis.

Liver tissues for molecular analysis

After removing the liver, around half a gram of tissue was placed in Eppendorf tubes. These tubes were promptly placed in liquid nitrogen and kept at -80 °C until the RNA was extracted to evaluate the expression of the following genes: miRNA-29, fibroblast growth factor (FGF), transforming growth factor-β1 (TGF-β1), and hepatocyte growth factor (HGF) (RT-PCR).

Liver specimens for histopathological examination

Following the procedures outlined by Bancroft and Gamble (2008), liver tissue samples were kept in 10% neutral buffered formalin solution.

Following adequate fixation, the specimens underwent dehydration using an ascending strength of ethyl alcohol, followed by clearing in xylol. Subsequently, the specimens were embedded in paraffin for precise blocking. For microscopy, these specimens were sectioned at a thickness of 5 µm and subjected to staining with hematoxylin and eosin (H and E).

2.5. Analysis

2.5.1. Biochemical analysis:

Serum activities of ALT and AST were assessed using the kinetic method outlined by Schuman et al. (2002), while alkaline phosphatase (ALP) activity was determined following the procedure described by Rosalki et al. (1993).

2.5.2. Molecular analysis:

The levels of mRNA expression of FGF2, TGF-β1, HGF, and miRNA-29 in the liver of rats were evaluated using real-time quantitative polymerase chain reaction analysis (real-time qPCR), as shown in Table 1. The control for loading was GAPDH. Following the directions given, total RNA was isolated from the liver using the High Pure RNA Isolation Kit (iNtRON Biotechnology, easy-REDTM Total RNA Extraction Kit). After that, the RevertAidTM First Strand cDNA Synthesis Kit was used for reverse transcription of the cDNA (Thermo Scientific, Fermentas, #EP0451, USA). The use of Faststart Universal SYBR Green Master for real-time quantitative PCR amplification was carried out (Roche, GER). The 2-ΔΔCt technique was used to normalize the expression of the target genes to glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (Livak and Schmittgen, 2001).

Liver miR-29 expression was measured in vivo using real-time PCR and SYBR Green (#K0221; Thermo Scientific, USA), with U6 serving as the internal control. Following the manufacturer's recommendations, the extracted cDNA was amplified using a universal reverse primer and a miRNA-specific forward primer (Quanti-Mir RT kit) (Table 2).

Table 1: Forward and reverse primers sequence for primers used in qPCR.

Gene	Forward primer (5'-----3')	Reverse primer (5'-----3')
FGF2	GAACCGGTACCTGGCTATGA	CCGTTTTGGATCCGAGITTA
TGFB1	AAGAAGTACCCCGGTGCTA	TGTGTGATGTCTTTGGTTTTGTCA
HGF	GAATGCATGACCTGCAACGG	TGTCGGGATATCTTCCGGC
GAPDH	CAACTCCCTCAAGATTGTCAGCAA	GGCATGGACTGTGGTCATGA

Table 2: Universal reverse primers sequence for primers used in qPCR.

Gene	Primer sequence (5'-----/3)
miRNA-29	TAGCACCATCTGAAATCGGTTA
U6	TGACACGCAAATTCGTGAAGCGTTC
Universal reverse primer	CCAGTCTCAGGGTCCGAGGTATTC

2.6. Statistical analysis.

The data were reported as means ± SEM employing SPSS software (Version 13.0, 2009). Duncan's multiple range test (DMRT) was utilized for individual comparisons, while statistical significance was assessed through the implementation of a one-way

analysis of variance (ANOVA). The statistical significance was established as $p \leq 0.05$.

3. RESULTS

Table (3) and Figure 1, indicated a notable increase in serum ALT, AST, and ALP activities in rats with DEN/CCL4-induced liver cancer compared to the standard control group. Treatment with ginger extract and ginger nanoparticles in DEN/CCL4-induced HCC led to a significant reduction in liver enzymes compared to the untreated group (G2), with the most substantial decrease observed in Group 4 (G4).

Table 3: Effect of GE or GNPs treatment on serum AST, ALT and ALP activities in DEN/CCL4-induced HCC in rats.

Animal groups	ALT (U/L)	AST (U/L)	ALP (U/L)
G1:Normal control	62.65±2.31 ^d	72.38±3.20 ^d	128.39±5.94 ^d
G2:DEN/CCL4	188.19 ±8.50 ^a	217.00±8.90 ^a	338.42±17.38 ^a
G3:DEN/CCL4 +GE	100.85 ±5.45 ^b	150.00±6.35 ^b	228.55±11.63 ^b
G4:DEN/CCL4 +GNPs	83.62±3.71 ^c	126.21±5.16 ^c	195.26±9.85 ^c

Data are presented as (Mean ± SEM). SEM = Standard error of mean. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

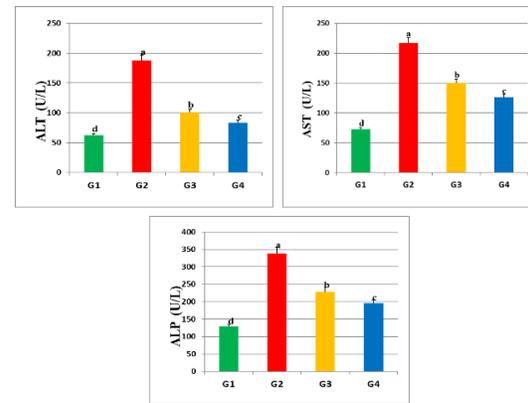


Figure 1: Effect of GE, GNP treatment on serum ALT, AST and ALP activities in experimental model of hepatocarcinogenesis in rats.

In the qPCR results, presented in Table (4) and Figure (2), there was a significant upregulation in the gene expression levels of TGFB1, FGF, and HGF in the liver tissue of rats with DEN/CCL4-induced liver cancer compared to the control group. However, this expression was significantly downregulated after treatment with ginger extract and ginger nanoparticles, with the most notable decrease observed in Group 4 (G4) compared to the non-treated DEN/CCL4 group.

Table 4: Treatment effect of GE, GNP on liver FGF2, TGF-β1 and HGF gene expressions in experimental model of hepatocarcinogenesis in rats.

Animal groups	FGF2 Fold change mean± SEM	TGFβ1 Fold change mean± SEM	HGF Fold change mean± SEM
Animal groups	1.00±0.00 ^d	1.00±0.00 ^d	1.00±0.00 ^d
G1:Normal control	11.08±0.56 ^a	3.56±0.12 ^a	7.78±0.36 ^a
G2:DEN/CCL4	7.16±0.35 ^b	2.17±0.1 ^b	3.71±0.14 ^b
G3:DEN/CCL4 +GE	3.86±0.14 ^c	1.57±0.08 ^c	1.85±0.11 ^c

Data are represented as (Mean± SEM). SEM = Standard error of mean. Mean values with different superscript letters in the same column are significantly different at (P≤0.05)

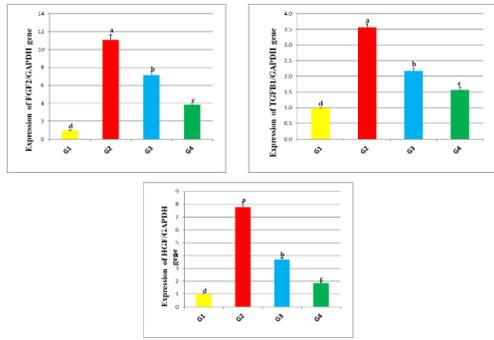


Figure 2: Effect of GE or GNPs treatment on liver , FGF2, TGF-β1 and HGF gene expressions in experimental model of hepatocarcinogenesis in rats.

Table (5) and Figure (3) illustrate a significant down-regulation in the gene expression of microRNA-29 in the liver tissue of rats with DEN/CCL4-induced HCC compared to the control group. Treatment with ginger extract (GE) or ginger nanoparticles (GNPs) in DEN/CCL4-induced HCC resulted in a significant upregulation of micro-RNA-29 gene expression, with the most pronounced increase observed in GNPs (G4) compared to the non-treated DEN/CCL4 group (G2).

Table 5: Effect of GE and GNP treatment on liver tissue microRNA-29 gene expression level in DEN/CCL4 induced HCC in rats.

Animal groups	microRNA-29 Fold change mean	SEM
G1:Normal control	1.00 ^e	0.00
G2:DEN/CCL4	0.44 ^d	0.03
G3:DEN/CCL4 +GE	4.86 ^b	0.21
G4:DEN/CCL4 +GNPs	6.54 ^a	0.3

Data are presented as (Mean ± SEM). SEM= Standard error of mean.

Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

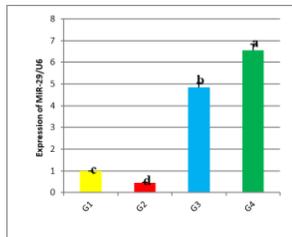


Figure 3: Effect of GE and GNP treatment on liver tissue microRNA-29 gene expression level in DEN/CCL4 induced HCC in rats.

Histopathological findings

In the control group, the liver displayed a histological appearance within the normal range, featuring intact central veins, portal areas, and hepatocytes (Fig. 4A). Contrastingly, in the livers of the DEN-treated group, there was evidence of multifocal hydropic degeneration of hepatocytes, characterized by swollen hepatocytes exhibiting pale vacuolated cytoplasm (Fig. 4B). Furthermore, occasional variably sized foci of lytic necrosis were observed, indicating a disruption of normal hepatic architecture and replacement by edema, hemorrhages, eosinophilic cellular material, and nuclear debris (Fig. 4C). Infrequently, coagulative necrosis of a few hepatocytes was present, characterized by a loss of cellular detail, shrunken hepatocytes with hyper eosinophilic cytoplasm, and pyknotic nuclei. Variable amounts of fibrous tissue proliferation were noted between the degenerated hepatocytes and in portal areas, along with proliferated

bile duct epithelium and lymphocyte infiltration (Fig. 4D). Clear cell foci of cellular alteration were observed, consisting of hypertrophied hepatocytes with vacuolated cytoplasm in a pale pink color (Fig. 5A). These foci seamlessly merged with the surrounding hepatic parenchyma, showing no separation or fibrous connective tissue capsule. Occasionally, there were eosinophilic foci of hepatocytes with hyperchromatic, variably sized, and centrally located nuclei (Fig. 5B). The liver of the DEN-Ginger treated group revealed a normal hepatic architecture with multifocal areas of moderate hydropic degeneration of hepatocytes (Fig. 5C), mild biliary hyperplasia, and lymphocytic infiltration in portal areas. Rarely, there was coagulative necrosis in a few hepatocytes. The liver of the DEN-Nano Ginger treated group revealed a normal hepatic architecture manifested as pale vacuolated cytoplasm and a modest degeneration of hepatocytes (Fig. 5D).

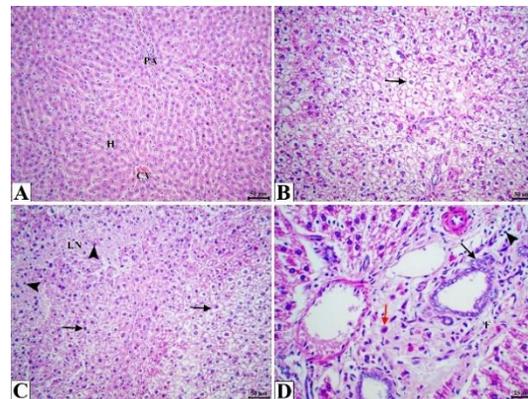
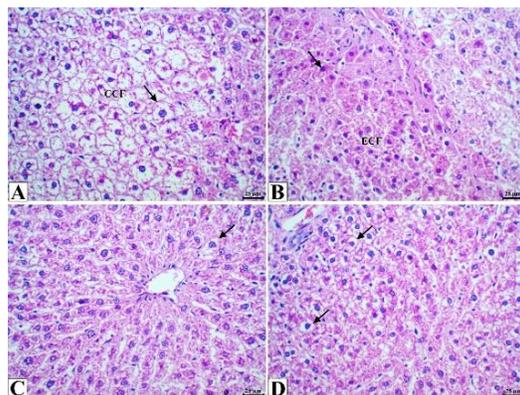


Figure 4: Liver sections of rats from control (A) and DEN-treated (B - D) groups. (A) Normal histological appearance of hepatic architecture, portal area, central vein and hepatocytes. (B) Marked hydropic degeneration of hepatocytes characterized by swollen pale vacuolated cytoplasm (arrow). (C) Focal lytic necrosis characterized by loss of normal hepatic architecture; note degeneration (arrow) and coagulative necrosis (arrowhead) of surrounding hepatocytes. (D) Moderate strands of fibrous tissue proliferation in portal area along with proliferated bile duct epithelium, (black arrow) necrotic hepatocytes (red arrow) and lymphocytes (arrowhead) infiltrate. H: hepatocytes, PA: portal area, CV: central vein, LN: lytic necrosis, and F: fibrous tissue. H&E stain

Figure 5: Liver sections of rats from DEN (A, B), DEN-Ginger (C) and DEN-



NanoGinger (D) treated groups. (A) Clear cell focus of hepatocellular alteration composed of hypertrophied hepatocytes with pale vacuolated cytoplasm (arrow) and enlarged nuclei; note the focus merge imperceptibly with surrounding hepatic parenchyma without separation. (B) Eosinophilic cell focus with hyperchromatic (arrow) centrally located nuclei. (C) Normal hepatic architecture with moderate hydropic degeneration (arrow) of hepatocytes. (D) Normal hepatic architecture with mild hepatocytes degeneration characterized by swollen pale vacuolated cytoplasm (arrow). CCF: clear cell focus and ECF: eosinophilic cell focus. H&E stain

4. DISCUSSION

Serum indicators of liver damage, specifically ALT, exhibited an increase in livers affected by HCC. This rise suggests the progression of carcinogenesis and indicates liver damage with the initiation of preneoplastic lesions (Hamza et al., 2021). The current findings reveal a significant elevation in serum ALT, AST, and ALP activities in rats with DEN/CCL4-induced liver cancer. These results align with the observations of Jin et al. (2013), who noted a substantial increase in AST, ALT, and ALP activities after administering DEN.

Treatment with ginger extract and ginger nanoparticles in DEN/CCL4-induced HCC resulted in a significant decrease in liver marker enzymes compared to the non-treated group. These outcomes are consistent with the findings of Attyah and Ismail (2012), who demonstrated that ginger extract reduced the elevated activity of serum ALT and AST compared to the group subjected to cisplatin. These improvements could be attributed to the presence of ginger components stabilizing the plasma membrane of hepatocytes and impeding the release of ALT and AST into the extracellular fluid. Moreover, the supplementation of ginger extract with diazinon-intoxicated rats can alleviate serum AST, ALT, and ALP activities, indicating that exogenous antioxidants could protect liver function (Elshafae et al., 2023).

The liver tissue of rats with DEN/CCL4-induced liver cancer exhibited an elevated expression of TGF- β 1, FGF2, and HGF genes. This heightened expression was significantly downregulated following treatment with ginger extract and ginger nanoparticles compared to the DEN untreated group. TGF- β 1, as a multifunctional polypeptide, influences tumor cell behavior by directly binding to TGF- β 1 receptors on tumor cells or affecting the pre-tumor environment. It promotes angiogenesis, increasing tumor vascularity, a process that can be impeded by TGF- β 1 neutralizing antibodies (Ito et al., 1995). Additionally, TGF- β 1 acts as a potent suppressor of immune function, potentially inhibiting the immunological mechanisms crucial for tumor identification and cytolysis (Massague, 1990). Similarly, Mansour et al. (2010) noted that treatment with ginger showed a marked decrease in the hepatic level of TGF- β 1 compared with the DEN-CCL4 group. The significant decrease in FGF2 gene expression observed in liver tissue following treatment with ginger extract and ginger nanoparticles aligns with the findings of Shammugam et al. (2022). Who explained that in both in vivo and in vitro studies, gingerol inhibits the growth of human vascular endothelial cells and counteracts the angiogenic response induced by FGF2. This suggests the potential utility of gingerol as an antiangiogenic and anticancer agent against various cancers by suppressing angiogenesis. This suggestion corresponds with the findings of Vijaya Padma et al. (2007), who revealed that 6-gingerol inhibits basic fibroblast growth factor (b-FGF)-induced proliferation, causing cell cycle arrest in G1 in human endothelial cells. Likewise, Mansour et al. (2010) demonstrated

that treatment with ginger showed a marked decrease in the hepatic level of FGF2.

Ginger is a plant of the family Zingiberaceae, including curcumin in its composition (Mbadiko et al., 2020). Zhang et al., (2017) elucidate that curcumin exhibits a suppressive effect against the angiogenic factor FGF2. HGF has an essential role during liver development (Schmidt et al., 1995). Although Tsunoda et al., (1998) reported that HGF participates in hepatocarcinogenesis, its role is not yet determined. HGF may also suppress or enhance hepatocarcinogenesis (Tsunoda et al., 1998; Persnell et al., 1998).

The existing results showed significant upregulation of HGF expression in the liver tissue of DEN/CCL4 untreated rats. Similarly, Abdelgawad and Ghareeb, (2010) demonstrated an increase in HGF in the serum of rats that were given DEN orally. In the current study, treatment with GE or GNPs for DEN/CCL4-induced HCC in rats exhibited downregulation of HGF, this result reflects the crucial role of ginger and ginger nanoparticles as anti-angiogenic factors during hepatocarcinogenesis. The obtained results are in concordance with (Zhang et al., 2017) who mentioned that curcumin exhibited a suppressive effect against the angiogenic factor HGF.

The DEN/CCL4 group exhibited a significant reduction in the expression of the miRNA-29 gene. The miR-29 family, encompassing miR-29a, miR-29b, and miR-29c, displays varied expression across liver cell types, with miR-29b exhibiting notably higher expression in hepatic satellite cells (HSCs) compared to sinusoidal endothelial cells, hepatocytes, and Kupffer cells (Rederburg et al., 2011). In the context of HCC, miR-29a/b/c is downregulated in human HCC tissues, and this decreased expression is associated with poor patient survival. (Parpert et al., 2014). Functional studies have indicated that miR-29b can hinder tumor formation and reduce tumor size in HCC (Xiong et al., 2010). Additionally, miR-29a/b/c promotes apoptosis of HCC cells by targeting cell survival genes, specifically myeloid cell leukemia-1 (MCL-1) and B cell leukemia-2 (Bcl-2). This tumor-suppressive role positions miR-29 as a potential prognostic marker and therapeutic target for HCC (Wang et al., 2020). The downregulation of hepatic miR-29a/b/c is also observed in advanced fibrosis, and its restoration has shown promise in ameliorating liver fibrosis induced by CCL4 (Matsumoto et al., 2016). Notably, (Wu et al., 2020) mentioned that the expression of miR-29c-3p, a specific member of the miR-29 family, was diminished in tissues and cell lines of HCC. Treatment with ginger extract (GE) and ginger nanoparticles (GNPs) resulted in a significant increase in miRNA-29 expression, with GNPs exhibiting the highest enhancement. There is evidence that the excessive expression of miR-29c-3p inhibits the apoptosis, proliferation, migration, and in vivo expansion of HCC tumors. This suppression is mediated through negative regulation of DNA methyltransferases 3B (DNMT3B) and the large tumor suppressor (LATS1)-associated Hippo-YAP/TAZ signaling pathway. Based on these results, it appears that miR-29c-3p functions as an HCC tumor suppressor

and represents a potential therapeutic target for hepatocarcinogenesis (Ruiz et al., 2022).

5. CONCLUSIONS

In conclusion, GE and GNPs may act as chemotherapeutic agents by noticeably activating the expression of tumor suppressor gene miRNA-29 and suppressing angiogenesis growth factors in rats hepatocarcinogenesis described by progressive cellular and molecular dedifferentiation of hepatocytes.

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