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Potential therapeutic and apoptotic impact of ginger and ginger nanoparticles against liver preneoplastic lesions development by inhibition of oncogenic miRNA-221 and initiation of Bcl-2/ caspase 3 signaling pathways

Noha N. Mohamed¹, Samy A. Hussein¹, Yakout A. El- Senosi¹, Mahmoud A. Emam², Shawky A. Moustafa³

¹ Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Benha University, Egypt

² Department of Histology, Faculty of Veterinary Medicine, Benha University, Egypt

³ Department of Pathology, Faculty of Veterinary Medicine, Benha University, Egypt.

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ABSTRACT

Hepatocellular carcinoma (HCC) is the second most fatal cancer, caused by either factors outside the body or even with hereditary or genetic changes. The potential chemotherapeutic effect of ginger extract (GE) and ginger nanoparticles (GNPs) against diethylnitrosamine (DEN) and carbon tetrachloride (CCl₄) induced HCC in rats was evaluated. HCC was induced via utilizing DEN injection (200 mg/kg b. wt/ I.P), then 2 weeks later of DEN injection rats received 3 weekly successive doses of CCl₄ diluted with corn oil at a ratio of 1:1(3ml/kg b.wt) orally to boost the carcinogenic impact. The administration of DEN and CCl₄ was repeated after a period of 5 weeks. 15 weeks after HCC induction, treatment with GE (300mg/kg b.wt/day) and GNPs (50mg/kg b.wt/day) were given orally and continued for six weeks. Twenty-four male rats were separated into four equal groups. Group 1 (normal control): Rats received saline as a vehicle, Group 2: (DEN/CCl₄ induced HCC), Group 3: (DEN/CCl₄ +GE), and Group 4: (DEN/CCl₄ +GNPs). The results revealed significant upregulation in liver microRNA-221 with obvious down-regulation of Nrf2 and Bcl-2 and insignificant downregulation in caspase 3 gene in HCC-induced rats. GE and GNPs treatment exhibited a significant decrease in liver marker enzymes with downregulation of microRNA-221 and upregulation of Nrf2, Bcl-2 and caspase 3 gene expression. These findings suggested that GE and GNPs have a beneficial therapeutic effect against liver cancer, inhibiting growth-promoting oncogene and increasing apoptosis.

1. INTRODUCTION

Liver cancer ranks as the third most major contributor to cancer-related mortality worldwide and the fifth most prevalent malignancy (Kung et al., 2010). Over 80 % of initial malignant liver neoplasms are hepatocellular carcinomas (Satir et al., 2007). According to current understanding of cancer mechanisms, all cancers are environmental and genetic in nature, meaning they arise from a combination of factors such as external exposures and inherited or genetic abnormalities that effort together to generate the illness (Clapp, 2005). Ground water has high levels of nitrate, which is caused by N-Diethylnitrosamine (DEN), a nitrosamine that is known to cause liver cancer. A variety of products, including cosmetics, agricultural chemicals, pharmaceutical agents, cured and fried foods, and Cheddar cheese, include this compound (Mahmoud and Abdul- Hamid, 2012). Among the most easily replicable carcinogens is diethylnitrosamine, which is used to induce the HCC

model in rats. A single dosage of DEN, a carcinogen which induces mutagenesis, DNA methylation, and the biotransformation of healthy hepatocytes into nascent cells, may possibly result in the commencement of the HCC (Santos et al., 2017). CCl₄ is a hazardous substance that functions as a cleaning agent, degreaser, and chemical intermediate. CCl₄ damages liver tissue by causing fatty degeneration, fibrosis, and liver failure (Matsubara et al., 1983). The damage-causing mechanism of CCl₄ in tissues can be explained as oxidative damage caused by lipid peroxidation which starts after the conversion of CCl₄ to free radicals of highly toxic trichloromethyl radicals ($\bullet\text{CCl}_3$) and trichloromethyl peroxy radical ($\bullet\text{CCl}_3\text{O}_2$) via cytochrome P450 enzyme (Unsal et al., 2020). Ginger, scientifically known as *Zingiber Officinale* Rosco, is a member of the Zingiberaceae family. It serves as both a culinary spice and a medicinal herb, comprising a diverse array of phytochemical compounds, such as flavonoids and tannins, which exhibit anti-carcinogenic, anti-inflammatory,

antioxidant, and glycosidic properties. Ginger possesses antioxidant properties due to the gingerols, shogaols, zingerone, gingerdiol, and zingiberene that it contains (Akbari et al., 2019). Many clarify that ginger possesses hepatoprotective properties (Akbari et al., 2019) by enhancing liver function and biomarker levels, this substance reduces inflammation, boosts antioxidant activity, and inhibits lipid peroxidation induced by toxins.

Drug delivery systems by nanotechnology represented a hopeful promise to enhance cancer treatment (Xiao et al., 2015). These delivery vehicles improved permeability and retention effect, enabling drugs to reach more passively into leaky vasculatures around the mass (Liu et al., 2016). This approach has limits in this passive targeting depending on the degree of vascularization and angiogenesis of tumor (Singh et al., 2016). The objective of the current research was therefore to ascertain the potential therapeutic impact of ginger and ginger nanoparticles in experimental model of HCC in rats by means of assessing some epigenetic and molecular markers in conjunction with histopathological investigation in hepatic tissues.

2. MATERIALS AND METHODS

2.1. Experimental animals

Thirty-two male albino rats, weighing between 100 and 150 g at 4-5 weeks of age, used in this study and purchased from the laboratory animals research center, faculty of veterinary medicine, Benha university. Rats were kept in individual wire-mesh cages with 12 hours of light and dark cycles, enough ventilation, and humidity. An unlimited supply of clean, fresh water is given to the animals, along with regular pellet feed. Rats are left for 15 days before the experiment for adaptation. The Experimental protocol was conducted according to the guide for Institutional Animals Care and Use Committee and approved by Research Ethics Committee, Faculty of Veterinary Medicine, Benha University (BUFVTM 14-03-23)

2.2. Chemicals and natural agents:

The following substances were employed in the current study as chemicals and natural agents:

2.2.1. Diethylnitrosoamine (DEN) with common name (N-Nitrosodiethylamine):

A 1g/1ml vial of N-nitrosodiethylamine, which is a transparent yellow liquid, was acquired from Sigma Aldrich Company, an Egyptian distributor of pharmaceuticals, chemicals, and medical devices. A solution of newly produced DEN in normal saline was administered intraperitoneally to rats at a weight of 200 mg/kg b.wt (Singh et al., 2009).

2.2.2. Carbon Tetrachloride (CCl₄):

Carbon tetrachloride present in colourless liquid form and purchased from El- Gomhouria Company for Trading Chemicals and Medical Appliances, Egypt. CCl₄ was freshly prepared in corn oil at (1:1) dilution and orally administered to rats at a dose of (3ml/kg b.wt) 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 were dried for 24 hours at a temperature range of 50-60°C in an oven. The ginger powder was extracted through maceration utilizing distilled water as the solvent after dried ginger was ground with a mechanical mixer (The powder to the water ratio was 1:10). The extraction was conducted for twenty-four hours at 75°C. In addition, the solid waste was subjected to filtration by the utilization of filter paper to isolate the filtrate or extract. The ginger extracts were kept at 8°C in dark amber bottles. (Manuhara et al., 2018). Ginger extract was administered orally at a dose of (300 mg/kg b.wt/day) for six weeks (Bakr 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 synthesis of chitosan nanoparticles was carried out via ionotropic gelation. (Hasanin et al., 2018). An aqueous solution of tripolyphosphate (TPP) was introduced into a solution of chitosan to generate blank nanoparticles. To obtain a homogeneous solution, 1 gm of Chitosan powder was dissolved in 200 ml of 1% acetic acid (pH = 4) and agitated for 6 hours. Following this, 150 ml of 0.2% w/v TPP were added drop by drop. Following the transformation of the clear solution to turbid, which indicated the development of CSNPs, the suspension underwent three DH₂O washes and 30 minutes of centrifugation at 12000 rpm (Hermle Z32 HK, Germany).

b-Preparation of Ginger Encapsulated Chitosan nanoparticles (GNPs):

The prepared ginger extract was dissolved in chitosan solution as previously mentioned with little modification. Briefly, 1g of ginger extract was dissolved in chitosan solution in weight ratio 10% then, for 20 minutes, tripolyphosphate was gradually added to the mixture while magnetic stirring was in place. After that, centrifugation was used to separate the chitosan nanoparticles for 30 minutes at a temperature of 4°C and a speed of 12,000 g. For a duration of six

weeks, ginger nanoparticles (GNPs) were delivered orally at a dosage of 50 mg/kg b.wt/day (Elrahman et al., 2020).

Induction of Hepatocarcinogenesis:

Hepatocellular carcinoma in rats was generated by administering DEN in normal saline at a dose of 200 mg/kg b. wt. via I.P injection (Singh et al., 2009), then 2 weeks later of DEN injection rats received 3 weekly successive doses of CCl₄ (3ml/kg b.wt) orally at 1:1 dilution in corn oil as a promoter of carcinogenic effect. A further DEN and CCl₄ injection were administered five weeks subsequent to the initiation of the DEN injection (Hassan et al., 2014). 15 weeks after HCC induction, therapeutic intervention with ginger extract and ginger nanoparticles was given and continued for six weeks.

2.3. Experimental design:

The rats were allocated into four basic equal groups, with each group consisting of 14 rats: Group 1 (Normal control): Rats were injected IP with saline as a vehicle for the duration of the 21-week (the experimental period).

Group 2 (DEN/ CCl₄): Rats developed HCC after receiving an I.P. injection of DEN in normal saline (200 mg/kg b.wt) and, 2 weeks later, CCl₄ diluted at a 1:1 ratio in corn oil was administered orally as a promoter of the carcinogenic effect. DEN and CCl₄ injections were repeated 5 weeks subsequent to the initial DEN injection.

Group 3 (DEN/ CCl₄ + GE): Rats were received DEN and CCl₄ injection as in group 2 and post-treated with GE (300 mg/kg b.wt/day) orally after 15 weeks from the administration of DEN and CCl₄ for 6 weeks.

Group 4 (DEN/ CCl₄ + GNPs): Rats were received DEN and CCl₄ injection as in group 2 and post-treated with GNPs (50 mg/kg b.wt/day) orally after 15 weeks from the administration of DEN and CCl₄ for 6 weeks. To ensure a consistent dose per kg body weight of rats for each group throughout the duration of the trial, the dosage was modified weekly during the experimental period in response to any changes in body weight.

2.4. Sampling:

2.4.1. Blood samples:

Blood samples obtained through ocular vein puncture into tubes with screw caps were subjected to centrifugation at 3000 r.p.m. for 15 minutes. For the determination of liver marker enzymes, the serum was obtained in a dry sterile sample tube via automated pipettes and thereafter kept in deep freeze at -20 °C until use.

2.4.2. Tissue specimens:

Rats were euthanized following Animal Ethics Committees at the end of the trial (21 weeks), after which the abdomen was cut open and the liver was extracted.

a- Molecular study:

For molecular study, livers were removed, and about 0.5 g kept in Eppendorf tubes, and immediately kept in liquid nitrogen and stored at -80°C till RNA extraction for determination of Nuclear factor erythroid2-related factor2 (Nrf2), B-cell lymphoma 2 (Bcl-2), Cysteine-aspartic acid protease (Caspase3) and miRNA -221 gene expression by reverse transcription polymerase chain reaction (RT-PCR).

b- Histopathological examination:

Histopathological examination of liver tissue specimens treated in 10% neutral buffered formalin solution (Bancroft and Gamble, 2008). Following appropriate fixation, the specimens underwent dehydration in increasing grades of ethyl alcohol, were subsequently cleared with xylol, encased in paraffin, and finely blocked. 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. Molecular analysis:

Real-time quantitative polymerase chain reaction analysis (real-time qPCR) was employed to determine the rat liver's mRNA expression levels of Nrf2, Caspase3, Bcl-2, and miRNA-221 (Table 1). GAPDH was implemented as a control for loading. Hepatic total RNA was extracted utilizing the High Pure RNA Isolation Kit (iNtRON Biotechnology, easy-REDTM Total RNA Extraction Kit) in accordance with the instructions of the manufacturer. cDNA was reverse-transcribed from each sample utilizing a RevertAidTM First Strand cDNA Synthesis Kit (Thermo Scientific, Fermentas, #EP0451, USA). Then, Faststart Universal SYBR Green Master was utilized to do real-time quantitative PCR amplification (Roche, GER). The 2-ΔΔCt technique was employed to normalize the target gene with glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (Livak and Schmittgen, 2001).

The quantification of microRNA-221 expression in the liver was achieved by employing real-time PCR and SYBR Green, with U6 serving as an internal control (Thermo Scientific, USA, #K0221), a miRNA specific forward primer (Table 2), and the quanti-Mir RT kit-supplied universal reverse primer was utilized to amplify the isolated cDNA per the manufacturer's guidelines.

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

Gene	Forward primer (5' ----- 3')	Reverse primer (5' ----- 3')
Caspase3	GGTATTGAGACAGACAGT GG	CATGGGATCTGTTTCTT TGC
Bcl-2	ATCGCTCTGTGGATGACT GAGTAC	AGAGACAGCCAGGAGA AATCAAAC
Nrf2	CACATCCAGACAGACACC AGT	CTACAAATGGGAATGTC TCTGC
GAPDH	CAACTCCCTCAAGATTGT CAGCAA	GGCATGGACTGTGGTCA TGA

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

Gene	Primer sequence (5' ----- /3')
miRNA-221	AACTGAACCTCCCTTTGA
U6	TGACACGCAAATTCGTGAAGCGTTC
Universal reverse primer	CCAGTCTCAGGTCCGAGGTATTC

2.6. Statistical analysis;

SPSS was utilized to represent all of the data as means ±SEM (Version 13.0, 2009). To determine statistical significance, one-way analysis of variance (ANOVA) was employed, while Duncan's multiple range test was utilized to facilitate comparisons between variables (DMRT). Values were deemed statistically significant when $p \leq 0.05$ (Steel et al., 1997).

3. RESULTS

The qPCR findings obtained are displayed in Table (3) and Figure (1) showed a significant down-regulation of Nrf2 and Bcl-2 with non-significant downregulation in caspase 3 gene expression level in liver tissue of DEN/CCl4-induced liver cancer in rats in contrast to the normal control group. This expression was significantly upregulated after treatment with ginger extract and ginger nanoparticles with the highest increase in the group (4) in contrast to DEN/CCl4 non-treated group. Table (4) and Figure (2) exhibited a significant upregulation of microRNA-221 gene expressions of liver tissue in DEN/CCl4-induced HCC in rats contrasted with the normal control group. Treatment with GE or GNPs to DEN/CCl4 induced HCC in rats demonstrated a significant downregulation of microRNA-221 gene expression with the greatest downregulation in GNPs (G4) contrasted with DEN/CCl4 non-treated group (G2).

Table 3: Effect of GE or GNPs treatment on liver tissue Nrf2, Caspase3 and Bcl-2 gene expression level in DEN/CCl4 induced HCC in rats.

Animal groups	Nrf2 Fold change mean± SEM	Caspase3 Fold change mean± SEM	Bcl-2 Fold change mean± SEM
G1:Normal control	1.00±0.00 ^a	1.00±0.00 ^c	1.00±0.00 ^a
G2:DEN/CCl4	0.10±0.01 ^d	0.99±0.09 ^c	0.10±0.01 ^d
G3:DEN/CCl4 +GE	0.59±0.04 ^c	5.90±0.26 ^b	0.36±0.02 ^c
G4:DEN/CCl4 +GNPs	0.78±0.05 ^b	11.79±0.5 ^a	0.64±0.04 ^b

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 ($P \leq 0.05$).

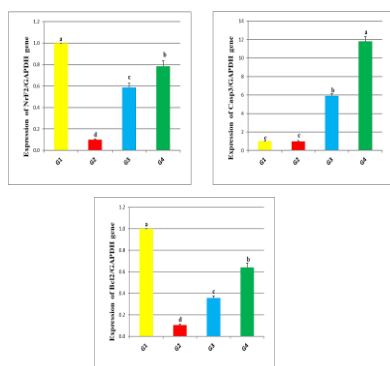


Figure 1: Effect of GE or GNPs treatment on liver Nrf2, Caspase3 and Bcl-2 gene expression in experimental model of hepatocarcinogenesis in rats.

Table 4: Effect of GE or GNPs treatment on liver tissue microRNA-221 gene expression level in DEN/CCl4 induced HCC in rats.

Animal groups	microRNA-221 Fold change mean	SEM
G1:Normal control	1.00 ^d	0.00
G2:DEN/CCl4	10.34 ^a	0.5
G3:DEN/CCl4 +GE	7.31 ^b	0.34
G4:DEN/CCl4 +GNPs	1.96 ^c	0.11

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 ($P \leq 0.05$)

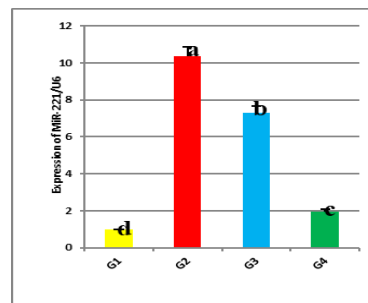


Figure 2: Effect of GE or GNPs treatment on liver miRNA-221 gene expression in experimental model of hepatocarcinogenesis in rats.

Histopathological findings

The liver of the control group demonstrated a normal histological appearance of hepatic architecture, portal areas, central veins, sinusoids and hepatocytes (Fig. 3A). The hepatocytes, appeared as large polygonal cells with eosinophilic cytoplasm and central spherical nuclei, were arranged as radial cords from the central vein. Meanwhile, livers of the DEN-treated group revealed multifocal degenerated changes of hepatocytes defined by swollen hepatocytes with pale vacuolated cytoplasm (hydropic degeneration), or few large, discrete, clear, cytoplasmic vacuoles that displace the nucleus (lipid-type degeneration). Occasional foci of lytic necrosis of varying sizes were observed; these foci were distinguished by the absence of proper hepatic architecture and the discontinuity of hepatic cords, replaced by edema admixed with fibrin, few hemorrhages, aggregates of eosinophilic cellular and karyorrhectic debris (Fig. 3B). Within these foci, remaining identifiable hepatocytes were necrotic, shrunken with pyknotic nuclei. Rarely, there was coagulative necrosis of few hepatocytes distinguished by the absence of cellular detail, reduced hepatocytes with hypereosinophilic cytoplasm and pyknotic nuclei. Moderate strands of fibrous tissue proliferation contain a few number lymphocytes, moderate amount of brown hemosiderin pigments and rich in congested capillaries (Fig. 3C) were observed in between the degenerated hepatocytes. The portal areas were expanded by moderate fibrosis that extended between portal areas and contained proliferated bile ductal epithelium admixed with lymphocytes, plasma cells, and neutrophils (Fig. 3D). Multifocally, clear cell foci of cellular change were observed, consisting of hypertrophied hepatocytes that seamlessly merged with the adjacent hepatic parenchyma; no separation or fibrous connective tissue capsule was present. The hepatic cells contained inside the foci exhibited enlargement, polygonality, vacuolated cytoplasm in a

pale pink hue, an enlarged nucleus positioned centrally, and conspicuous nucleoli (Fig. 4A). Occasionally, there were eosinophilic foci of cellular alteration consisting of polygonal hepatocytes with hyperchromatic, variably sized, and centrally located nuclei (Fig. 4B). The liver of DEN- Ginger treated group revealed maintained normal hepatic architecture with multifocal areas of moderate hydropic degeneration of hepatocytes (Fig. 4C), mild biliary hyperplasia and

lymphocytic infiltration in portal areas. Rarely, there were dilated and markedly congested sinusoids, with coagulative necrosis of few hepatocytes (Fig. 4D). The liver of DEN- NanoGinger treated group revealed maintained normal hepatic architecture with mild degradation of hepatocytes (Fig. 4E) distinguished by swollen pale vacuolated cytoplasm and dilated sinusoids (Fig. 4F).

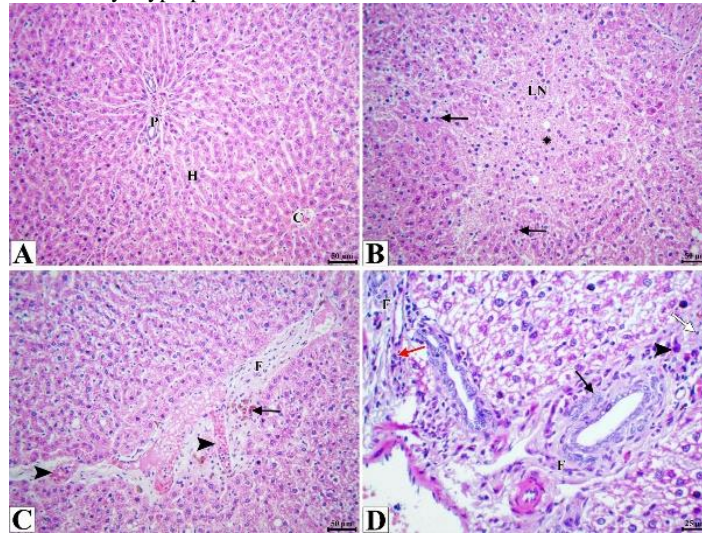


Figure 3: Liver sections of rats from control (A) and DEN-treated (B - D) groups. (A) Normal histological appearance of hepatic architecture, portal areas, central veins and hepatocytes. (B) Focal lytic necrosis defined by a loss of normal hepatic architecture with discontinuity of hepatic cords, replaced by edema (asterisk) admixed with fibrin, few hemorrhages, and cellular debris; note necrotic hepatocytes with pyknotic nuclei (arrow). (C) Moderate strands of fibrous tissue proliferation. In between the degenerated hepatocytes, contain lymphocytes, hemosiderin pigments (arrow) and congested capillaries (arrowhead). (D) Mild fibrosis in portal areas contain proliferated bile duct epithelium (black arrow) admixed with lymphocytes (white arrow), plasma cells (arrowhead), and neutrophils (red arrow). H: hepatocytes, P: portal area, C: central vein, F: fibrous tissue and LN: Lytic necrosis.

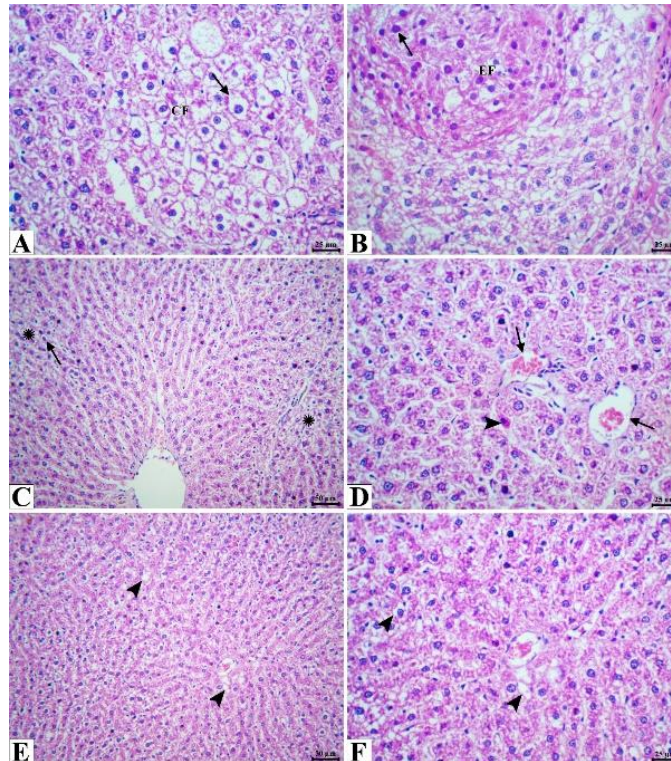


Figure 4: Liver sections of rats from DEN (A, B) DEN-Ginger (C, D) and DEN-Nano Ginger (E, F) treated groups. (A) Clear cell focus of hepatocytic alteration composed of hypertrophied hepatocytes with pale vacuolated cytoplasm (arrow), enlarged nucleus, and prominent nucleoli. (B) Eosinophilic focus of hepatocytic alteration composed of polygonal hepatocytes with hyperchromatic, variably sized, and centrally located nuclei (arrow). (C) Multifocal areas (asterisk) of moderate hydropic degeneration (arrow) of hepatocytes. (D) Mild congestion (arrow) with coagulative necrosis (arrowhead) of a few hepatocytes. (E) Normal hepatic architecture with mild degeneration (arrowhead) of hepatocytes. (F) Mild hepatocytes degeneration characterized by pale vacuolated cytoplasm (arrowhead). CF: clear cell focus and EF: eosinophilic focus.

4. DISCUSSION

In rats with DEN/CCl₄-induced liver cancer, the expression of the caspase 3 gene was not significantly downregulated, although Nrf2 and Bcl-2 were significantly downregulated. The expression in question exhibited a substantial increase in expression following treatment with ginger extract and nanoparticles, in comparison to the untreated group. Mahmoud et al., (2017) elucidate that 78 % reduction in hepatic Nrf2 was seen following seven days of dietary nitrosamine treatment. Also, Mahmoud et al., (2017) mentioned that DEN is shown to downregulate Nrf2 in the liver along with oxidative stress, inflammation, and angiogenesis induction.

Zerumbone, a constituent of Asian ginger oil, increases the expression of phase II detoxifying enzymes and the nuclear localization of antioxidant response element (ARE), which serves as both an indication and regulator of oxidative stress (Nakamura et al., 2004). Ginger extract induces an anti-inflammatory response by increasing the production of heme oxygenase-1 (HO-1), which in turn inhibits the NF- κ B signalling pathway, via upregulation of Nrf2 (Chi et al., 2015) by introducing a novel perspective on cancer prevention via the Nrf2/ARE pathway upregulation induced by ginger intake. The catalyst for the activation of protective genes' transcription is the antioxidant responsive element (ARE), a cis-acting component. A binding occurs between the transcription factor Nrf2 and the ARE. This mechanism, when activated, safeguards cells against oxidative stress-induced cell death. An enhancer element, the antioxidant responsive element (ARE) is responsible for stimulating the transcription of a battery of genes that encode phase II detoxification enzymes. This suggestion are in accordance with (Nakamura et al., 2004) who mentioned that zerumbone (a tropical ginger sesquiterpene) activates the Nrf2 dependent pathway. Also, Bak et al., (2012) elucidated that by stimulating Nrf2, a ginger extract high in shogaol might bolster antioxidant defense mechanisms.

Cancer is a pathological state defined by uncontrolled cellular proliferation and an imbalance between apoptosis and cell division. As a result of its involvement in apoptotic flux, caspase-3 deficiency may disrupt apoptosis, hence contributing to carcinogenesis. (Wong et al., 2011). Hence, the upregulation of caspase-3, as an apoptotic agent, has clinical importance (Wong et al., 2011). Yambel et al., (1999), shown that apoptosis could be induced in liver tumour cells via transduction of the human caspase-3 gene. The significant upregulation of caspase 3 gene expression in liver tissue after treatment with ginger extract and ginger nanoparticles are in accordance with those mentioned by (Annamalai et al., 2016) who stated that the prooxidant role of 6-shogaol upregulate caspase -3 expression. Likewise, (Nair et al., 2014) explain that ginger induce apoptosis by activation of caspase -3. Moreover, HU et al., (2012) demonstrated that, 6-shogaol impeded the development of tumours by

the stimuli of caspase- 3. Additionally, (Chen et al., 2007) recorded that ginger activate caspase- 3 resulting in DNA fragmentation.

The concept of apoptosis pertains to the programmed demise of cells within multicellular organisms, which can take place under both normal and abnormal physiological circumstances. There is either the internal mitochondrial pathway or the extrinsic death receptor pathway through which apoptosis takes place (Kroemer et al., 2007). The intrinsic mitochondrial pathway of apoptosis is associated with the activation of caspases, reprogrammed DNA synthesis (apoptosis), disruption of mitochondrial membrane permeability (apoptosis-induced protein release), inhibition of the anti-apoptotic effect of Bcl-2 and Bcl-x, and induction of p53, Bax, and Bak. The extrinsic death receptors pathway involves cellular membrane-localized receptors, such as Fas receptors, tumour necrosis factor (TNF) receptors, and TNF-related apoptosis-inducing ligand receptors. These receptors recruit adaptor proteins, one of which is initiator caspase-8. The activation of effector caspases (3), (6), and (7) ultimately results in cell apoptosis (Kroemer, et al., 2014).

Furthermore, Hemieda et al., (2019) stated that administration of ciprofloxacin in rats altered conditions in the apoptotic markers in the liver, the findings indicated significant decrease in the level of Bcl-2 and co-administration of ginger with ciprofloxacin attenuated the apoptotic activity of ciprofloxacin, as shown by significant increase in the level of Bcl-2 in respect to ciprofloxacin treatment alone. Similarly, Baiomy and Mansour (2011) reported that ginger increased the expression of Bcl-2 which may be due to the detoxification power of ginger against cadmium toxicity. On contrary, Wang et al., (2008) clarified that beta-Elementene, the major component of ginger decrease Bcl-2 expression. Also, Taha et al., (2010) established that Zerumbone at 30 and 60 mg/kg b wt. significantly reduced Bcl-2 expression in the cancerous hepatocytes.

MicroRNAs (miRNAs) assume a crucial role in numerous cellular biological processes by functioning as oncogenes or tumour suppressor genes (Deng et al., 2017). Cell proliferation, apoptosis, and differentiation are all molecular processes that are regulated by miRNAs (Zheng et al., 2013). A significant increase in the expression of microRNA-221 was detected in the liver of HCC induced with DEN/CCl₄. MiR-221 is expressed aberrantly in a number of malignancies, including HCC (Rong et al., 2013). Over expression of miR-221 is related with a more aggressive phenotype in HCC (Grameriante et al., 2009). In addition, miR-221 expression was elevated in HCC tumours relative to benign liver tissue that was free of illness (Pineau et al., 2009). In extremely aggressive HCC, miR-221 was among the most up-regulated of all miRNAs examined, according to the same investigators. Furthermore, the fact that miR-221 influences numerous pro-oncogenic pathways concurrently suggests that it may be a viable target for nonconventional therapies targeting HCC (Clin Cancer Res, 2009). Existing findings about the increase of microRNA-221 in the liver of HCC patients

are consistent with (Mackenze et al., 2014) who demonstrated that numerous types of messenger RNAs associated with the development and advancement of cancer have been found, and that oncogenic microRNAs (miRNAs), including miR-221, are frequently overexpressed in a variety of malignancies. Curcumin, an important ingredient of curcuminoids, was initially extracted from turmeric (*curcuma longa*), a member of the zingiberaceae (ginger) family that is also found in significant amounts in ginger (Jiang et al., 2017). Treatment with GE or GNPs to HCC-induced rats exhibited a significant downregulation of microRNA-221. Similarly, (Zhang et al., 2017) mentioned that curcumin causes downregulation of miR-221 due to its anti-angiogenic mechanism in HCC.

5. CONCLUSIONS

In conclusion, Ginger extract and Ginger Nanoparticles treatments could be possess therapeutic effect against DEN/CCL4 induced HCC. Also, these considered as natural apoptotic agents through enhancing of gene regulating apoptosis and regulation of growth promoting oncogenic miRNA-221.

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