The Carcinogenic Agent Diethylnitrosamine Induces Early Oxidative Stress, Inflammation and Proliferation in Rat Liver, Stomach and Colon: Protective Effect of Ginger Extract

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Abstract

Background: Diethylnitrosamine (DENA), a well-known dietary carcinogen, related to cancer initiation of various organs. The present study investigated the deleterious mechanisms involved in the early destructive changes of DENA in different organs namely, liver, stomach and colon and the potential protective effect of GE against these mechanisms.

Methods: Adult male albino rats were assigned into four groups. A normal control group received the vehicle, another group was injected with a single necrogenic dose of DENA (200 mg/kg, i.p) on day 21. Two groups received oral GE (108 or 216 mg/kg) daily for 28 days. Sera, liver, stomach and colon were obtained 7 days after DENA injection. Serum aspartate transaminase and alanine transaminase were detected as well as reduced glutathione (GSH), malondialdehyde, nitric oxide metabolites, interleukin 1β, tumor necrosis factor (TNF-α), alpha-fetoprotein (AFP) and nuclear factor-erythroid 2-related factor 2 (Nrf2) in liver, stomach and colon. Histopathological studies and immunohistochemical examination of cyclooxygenase-2 (COX2) were conducted. Results: DENA induced elevation in liver function enzymes with significant increase in oxidation and inflammation biomarkers and AFP while decreased levels of Nrf2 in liver, stomach and colon were detected. Histologically, DENA showed degenerative changes in hepatocytes and inflammatory foci. Inflammatory foci displayed increased expression of COX2 in immunohistochemical staining. GE-pretreatment improved liver function and restored normal GSH with significant mitigation of oxidative stress and inflammatory biomarkers compared to DENA-treated group. AFP was reduced by GE in both doses, while Nrf2 increased significantly. Histology and immunostaining of hepatic COX-2 were remarkably improved in GE-treated groups in a dose dependent manner. Conclusion: GE exerted a potential anti-proliferative activity against DENA in liver, stomach and colon via Nrf2 activation, whilst suppression of oxidation and inflammation.

Keywords: Diethylnitrosamine- ginger extract- oxidative stress- inflammation- proliferation- liver- stomach-colon- Rat
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Experimental design and treatment protocol

Animals

Forty adult male Wistar albino rats weighing 180-200 g were utilized in the present study. Standard food pellets and tap water were supplied ad libitum. Animals and food pellets were obtained from the animal house colony of the National Research Centre (NRC, Egypt). Animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA) and the experiment was conducted in accordance with ethical rules for standard experimental animal studies and the Medical Research Ethics Committee (MREC) of the National Research Centre under approval number: 15130.

Drugs and chemicals

Diethylnitrosamine (DENA) was purchased from Sigma-Aldrich (Germany). DENA was injected intraperitoneally in a single necrogenic dose of 200 mg/kg (Tessitore, 1998; Bansal et al., 2005). Ginger pure powder (Sigma), was suspended in 0.5% carboxymethylcellulose (CMC) in distilled water (vehicle). All other chemicals were of highest analytical grade available. All mandatory laboratory health and safety procedures have been complied with in the course of conducting the experimental work in this study.

Experimental design and treatment protocol

Animals were randomly allocated into four groups (10 rats each). Rats of the 1st group received 0.5% CMC and intraperitoneal injections of saline and served as normal control group. Group 2 received a single necrogenic dose of DENA (200 mg/kg, i.p)(Tessitore et al., 1996) on day 21 of the study. Groups 3 and 4 received oral GE (108 or 216 mg/kg/day), respectively, for 28 days during which a single necrogenic dose of DENA (200 mg/kg, i.p) on day 21 was injected. All animals were sacrificed 24 h after last GE treatment.

Serum biochemical analysis

Rats were anaesthetized with diethyl ether and blood samples were withdrawn from the retro-orbital venous plexus. Collected blood samples were allowed to stand for 10 min at room temperature then centrifuged at 4°C using centrifuge (Laborezentrifugen, 2k15, Sigma, Germany) at 3,000 r.p.m for 10 min. Sera were separated for assessment of levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using commercially available colorimetric assay kits (Biodiagnostic, Egypt) as previously described (Reitman and Frankel, 1957).

Hepatic, stomach and colon tissue biochemical analysis

Directly after blood sampling, rats were sacrificed by cervical dislocation under ether anesthesia. Liver, stomach and colon tissues were collected, washed in normal saline. The tissue was homogenized using MPW–120 homogenizer (Med instruments, Poland); the homogenate was centrifuged using a cooling centrifuge (Laborezentrifugen, 2k15, Sigma, Germany) at 4,000 r.p.m for 10 min. and the supernatant was assessed for hepatic, stomach and colon levels of reduced glutathione (GSH) (Beutler et al., 1963), lipid peroxides as malondialdehyde (MDA) (Mihara and Uchiyama, 1978) and nitric oxide (NOx) metabolites (Miranda et al., 2001). Moreover, inflammatory markers such as interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α) were assayed using ELISA kits (Hycult Biotech, Netherlands) and (RayBio, USA), respectively, according to the manufacturer’s instructions. Finally, alpha-fetoprotein (AFP) and nuclear factor-erythroid 2-related factor 2 (Nrf 2) were assessed using ELISA kits (KAMIYA BIOMEDICAL, USA, Cat. No. KT-59172) and (CUSABIO, China, Cat. No CSB-EQ027869RA), respectively, according to the manufacturer’s instructions.

Immunohistochemical analysis of cyclooxygenase-2 (COX-2)

For immunohistochemistry, 4 μm thick deparaffinized liver tissue sections were used. Briefly, deparaffinized liver slices were incubated overnight with the antibodies against COX-2 diluted 1:100. Endogenous peroxidase activity was blocked by incubation in 0.075% hydrogen peroxide in PBS. For antibody detection DAKO EnVision+ System, Peroxidase/DAB kit was employed. The sections were then counterstained with hematoxylin, dehydrated using graded alcohols and xylene, and mounted with Entelan. The immunostaining intensity and cellular localization of COX-2 was analyzed by light microscopy.

Histopathological examination

The other parts of liver tissues were fixed in 10% formalin buffered to pH 7.4, and processed for histopathologic examination as described previously (Sadik et al., 2008; Janani et al., 2010).
neutral buffered formalin and embedded in paraffin wax. Sections of 4 μm thick were stained with Hematoxylin and Eosin (H and E) and examined using binocular Olympus CX31 microscope (Bancroft et al., 1996).

### Statistical analysis
All values are presented as means ± standard error of the means (SEM) of eight experiments. Comparisons between different groups were carried out using one way analysis of variance (ANOVA) followed by Tukey’s multiple comparison post hoc test. Difference was considered significant when p<0.05. GraphPad prism® software version 6 for Windows (USA) was used to carry out these statistical tests.

### Results

#### Effect of ginger extract on liver function enzymes
Administration of single intraperitoneal dose of DENA (200 mg/kg) resulted in a significant elevation of liver function biomarkers. AST and ALT, were elevated significantly in DENA-treated rats compared to their normal counterparts. Pre-treatment of rats with ginger extract at 108 mg/kg/day, showed insignificant effect on either AST or ALT serum levels. While Pre-treatment of rats with ginger extract at 216 mg/kg/day, showed significant reduction in AST level reporting normal levels of AST in DENA- treated rats with insignificant effect on ALT serum levels (Figure 1).

#### Effect of ginger extract on liver, stomach, and colon tissues oxidative stress parameters
The GSH content in liver, stomach, and colon was significantly reduced following DENA administration in rats. Treatment of rats with ginger restored the normal GSH content in the liver and colon. However, normal level of stomach GSH was only observed in the group treated with the lower dose of ginger; in the rats treated with the high dose, the stomach GSH content was significantly lower than the normal and non-significantly different from that of rats treated with the lower dose of ginger.

### Table 1. Effect of Ginger Extract on Interleukin 1-Beta (IL-1β) Level in Liver, Stomach and Colon of DENA-Treated Rats

| Group             | Liver (IL-1β, pg/ml) | Stomach (IL-1β, pg/ml) | Colon (IL-1β, pg/ml) |
|-------------------|----------------------|------------------------|----------------------|
| Normal            | 316.5±22.7           | 144.2±5.7              | 184.6±15.47          |
| DENA              | 552.2±18.8           | 442.6±39.7             | 416.0±20.36          |
| DENA-Ginger (108mg/kg) | 390.2±3.7           | 249.8±18.1             | 269.0±11.46          |
| DENA-Ginger (216mg/kg) | 364.9±12.0           | 295.7±11.4             | 228.6±16.11          |

Control, rats treated with the vehicle and represented the normal group; DENA, rats treated with diethyl nitrosamine; DENA-Ginger (108 mg/kg), rats treated with diethyl nitrosamine and ginger (108 mg/kg/day); DENA-Ginger (216 mg/kg), rats treated with diethyl nitrosamine and ginger (216 mg/kg/day). Each value represents the mean ± standard error of the means (n=6). Statistical analysis was carried out using one-way ANOVA test followed by Tukey post hoc test. Significantly different from normal group at p<0.05. Significantly different from DENA group at p<0.05. Significantly different from the other DENA-Ginger group at p<0.05.

### Table 2. Effect of Ginger Extract on Tumor Necrosis Factor-Alpha (TNF-α) Level in Liver, Stomach and Colon of DENA-Treated Rats

| Group             | Liver (TNF-α, pg/ml) | Stomach (TNF-α, pg/ml) | Colon (TNF-α, pg/ml) |
|-------------------|----------------------|------------------------|----------------------|
| Normal            | 556.5±30.9           | 461.4±11.8             | 147.4±12.6           |
| DENA              | 1245.0±93.0          | 946.3±32.4             | 495.9±24.7           |
| DENA-Ginger (108mg/kg) | 879.3±29.3           | 699.7±46.8             | 279.6±23.7           |
| DENA-Ginger (216mg/kg) | 831.1±21.1           | 515.1±12.2             | 264.5±22.4           |

Control, rats treated with the vehicle and represented the normal group; DENA, rats treated with diethyl nitrosamine; DENA-Ginger (108 mg/kg), rats treated with diethyl nitrosamine and ginger (108 mg/kg/day); DENA-Ginger (216 mg/kg), rats treated with diethyl nitrosamine and ginger (216 mg/kg/day). Each value represents the mean ± standard error of the means (n=6). Statistical analysis was carried out using one-way ANOVA test followed by Tukey post hoc test. Significantly different from normal group at p<0.05. Significantly different from the other DENA-Ginger group at p<0.05.

### Table 3. Effect of Ginger Extract on Alpha-Fetoprotein (AFP) Level in Liver, Stomach and Colon of DENA-Treated Rats

| Group             | Liver (AFP, ng/g tissue) | Stomach (AFP, ng/g tissue) | Colon (AFP, ng/g tissue) |
|-------------------|--------------------------|-----------------------------|--------------------------|
| Control           | 13.30±0.49               | 2.00±0.13                   | 3.59±0.20                |
| DENA              | 154.57±5.26              | 11.61±0.60                  | 29.45±1.92               |
| DENA-Ginger (108mg/kg) | 70.14±1.89               | 6.22±0.21                   | 10.28±0.42               |
| DENA-Ginger (216mg/kg) | 49.67±1.58               | 3.72±0.21                   | 6.78±0.21                |

Control, rats treated with the vehicle and represented the normal group; DENA, rats treated with diethyl nitrosamine; DENA-Ginger (108 mg/kg), rats treated with diethyl nitrosamine and ginger (108 mg/kg/day); DENA-Ginger (216 mg/kg), rats treated with diethyl nitrosamine and ginger (216 mg/kg/day). Each value represents the mean ± standard error of the means (n=6). Statistical analysis was carried out using one-way ANOVA test followed by Tukey post hoc test. Significantly different from normal group at p<0.05. Significantly different from DENA group at p<0.05. Significantly different from the other DENA-Ginger group at p<0.05.
Figure 1. Effect of Ginger Extract on Liver Function Enzymes of DENA-Treated Rats. Control, rats treated with the vehicle and represented the normal group; DENA, rats treated with diethyl nitrosamine; DENA-Ginger (108 mg/kg), rats treated with diethyl nitrosamine and ginger (108 mg/kg/day); DENA-Ginger (216 mg/kg), rats treated with diethyl nitrosamine and ginger (216 mg/kg/day). a Significantly different from normal group at p <0.05; b Significantly different from DENA group at p <0.05; c Significantly different from the other DENA-Ginger group at p <0.05.

Figure 2. Effect of Ginger Extract Liver, Stomach and Colon contents of GSH (a), MDA (b), and NOx(c) of DENA-treated Rats. Control, rats treated with the vehicle and represented the normal group; DENA, rats treated with diethyl nitrosamine; DENA-Ginger (108 mg/kg), rats treated with diethyl nitrosamine and ginger (108 mg/kg/day); DENA-Ginger (216 mg/kg), rats treated with diethyl nitrosamine and ginger (216 mg/kg/day). a Significantly different from normal group at p <0.05; b Significantly different from DENA group at p <0.05; c Significantly different from the other DENA-Ginger group at p <0.05.
of DENA-treated group. On the other hand, a significant elevation of the liver, stomach, and colon MDA content was detected following DENA administration. Treatment of rats with both doses of ginger significantly retrieved the altered level of MDA in those organs’ tissues.

Moreover, a significant increase in liver, stomach, and colon content of NOx was observed in rats treated with DENA. Treatment of rats with the higher dose of ginger has been found to restore the normal levels of NOx and its effect was significantly better than that of the lower dose (Figure 2 a, b, and c).

**Effect of ginger extract on liver, stomach, and colon tissues pro-inflammatory cytokines; interleukin-1**

Liver, stomach, and colon content of the pro-inflammatory cytokine IL-1β was significantly reduced by DENA administration in rats. Treatment of rats with ginger significantly reduced IL-1β content in

![Image](https://via.placeholder.com/150)

**Figure 3.** Effect of GE on COX2 Immunoreactivity in Hepatic Tissues in DENA-Induced Injury. (a) Control group: showed no positive inflammatory foci and negative COX2 immunoreactivity. (b) DENA group: showed strong positive stain in the area of inflammation exhibited sever COX2 immunoreactivity. (c) GE (108 mg/kg) group: moderate immunoreactivity. (d) GE (216 mg/kg) group: mild COX2 immunoreactivity. (COX2, x400)

![Image](https://via.placeholder.com/150)

**Figure 4.** Effect of GE on the Histopathological Changes in the Hepatic Tissue in DENA-Induced Injury. (a) Normal control: showed normal architecture with the hepatocytes are normal run in thin plates (H), the portal areas showed normal structures with no fibrosis or inflammation (P). (b) DENA group: dense inflammatory cell infiltrate (I), areas of fibrosis (F), the hepatocytes are degenerated (H). (c) GE (108 mg/kg) group: degeneration of some of the hepatocytes (H), the cytoplasm appear foamy. Few foci of inflammation are still seen (I), some vessels are still dilated (C). (D) GE (216 mg/kg) group; showed better cytomorphology of the hepatocytes (H); with almost normal appearance of their cytoplasm. No foci of inflammation. No portal inflammation (P). The blood vessels showed mild dilatation and congestion (C). (H&E) (a and d x200; b and cx400).
the liver, stomach and colon by the two applicable doses. While the high dose of ginger significantly reduced both liver and colon IL-1β with no significant difference from normal group (Table 1). In addition, DENA administration significantly increased liver, stomach, and colon content of TNF-α, while pretreatment with ginger extract for four weeks significantly reduced TNF-α compared to DENA-treated rats. Ginger extract, at 216mg/kg, significantly reduced TNF-α in stomach of DENA-treated animals compared to the other dose of ginger (108mg/kg) (Table 2).

**Effect of ginger extract on liver, stomach and colon tissues alpha-fetoprotein (AFP)**

Alpha-fetoprotein (AFP) was significantly increased after DENA single intraperitoneal injection in liver, stomach and colon of rats compared to normal rats. Pretreatment with ginger extract significantly inhibited the dramatic elevation in AFP after DENA injection in liver, stomach and colon. Moreover, ginger extract at, 216 mg/kg, exerted significant reduction in AFP compared to the other dose of the extract in both liver and stomach, while restored normal levels of AFP in the colon of DENA-treated rats (Table 3).

**Effect of ginger extract on liver, stomach and colon nuclear factor-erythroid 2-related factor 2 (Nrf2)**

Diethylnitrosamine (DENA) in a single intraperitoneal injection (200 mg /kg) significantly decreased nuclear factor-erythroid 2-related factor-2 (Nrf2) in liver, stomach and colon of rats compared to normal rats. Pretreatment with ginger extract significantly inhibited the reduction in Nrf-2 level of liver, stomach and colon compared DENA group. Moreover, ginger extract, at 216 mg/kg, exerted significant elevation in Nrf-2 level of liver, stomach and colon tissues compared to the other dose of the extract (Table 4).

**Immunohistochemical and histopathological assessment of hepatic tissues**

Immunohistochemical assessment of hepatic cyclooxygenase-2 (COX2) revealed strong immunoreactivity of COX2 in hepatic tissues on week after 200 mg of DENA intraperitoneal injection. Meanwhile, administration of GE at 108 mg/kg showed moderate COX2 immunoreactivity compared to GE at 216 which displayed mild COX2 immunoreactivity (Figure 3).

Histopathological examination of liver tissues revealed dense inflammatory cell infiltrate areas of fibrosis and degeneration of hepatocytes after DENA injection. GE (108 mg/kg) exerted slight improvement while degeneration of some of the hepatocytes, foamy cytoplasm and inflammation are still seen. GE (216 mg/kg) showed better cytomorphology of the hepatocytes with almost normal appearance of their cytoplasm, no foci of inflammation, No portal inflammation, and blood vessels showed mild dilatation and congestion with significant improvement in the overall hepatic histopathological picture (Figure 4).

**Discussion**

Being one of the most frequently and heavily consumed natural dietary component, ginger and its polyphenolic compounds; zingerone, [6]-gingerol, and shogaol; have been reported for their chemoprotective and antioxidant effects in carcinogenesis (Chung et al., 2001; Mohd-Yusof et al., 2002; Manju and Nalini, 2005). Having more profound effect than gingerols, its active components, (Mukkavilli et al., 2014); researchers highlight the importance of utilizing entire ginger extract over its active components (Prasad and Tyagi, 2015b) with recently reported anti-oxidant, anti-inflammatory and anti-apoptotic effects of whole ginger rhizome extract in rat model of diabetic nephropathy (Al Hroob et al., 2018).

Nitrosamines as dietary carcinogens are associated with the etiology of HCC and contribute to the development of oxidative stress, chronic inflammation, and cellular proliferation in response to tissue injury, leading to hepatocarcinogenesis (Darvesh and Bishayee, 2013).

Both environmental and N-nitrosamines born-food hold a health hazard for human and animals. Experimentally, DENA is used to investigate its cytotoxic mechanisms on different tissues and organs. Moreover, DENA causes alterations in serum and tissue enzyme markers (Atakisi et al., 2013). The International Agency for Research on Cancer (IARC) categorized DENA as a “probable carcinogenic to humans” (category A2) (IARC, 1987). The catalysis of DENA by cytochrome P-450-dependent enzymes of monooxygenase system yields active metabolite ethyl radical that covalently binds to DNA leading to cellular necrosis, mutation and cancer (Skog, 2002). Oxidative stress-induced cell injury plays a crucial role in DENA-induced carcinogenesis (Bansal et al., 2000).

Liver-specific enzyme markers, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT), are released during hepatocytes’ injury (Galle et al., 2014). AST, ALT and ALP are considered more sensitive parameters to assess liver injury in rodent species (Galle et al., 2014). Previous studies indicated that DENA-induced liver injury is accompanied by elevated activities of these enzymes (Saidik et al., 2008; Sayed-Ahmed et al., 2010; Jin et al., 2013; Galle et al., 2014). In accordance with present data that clearly stated a significant elevation in ALT and AST enzyme activities after a single necrogenic dose of DENA, indicating established liver injury. Taking into consideration the hepatoprotective activity of ginger extract (GE) against several hepatotoxic agents (Atta et al., 2010; Abdel-Azeem et al., 2013; Shivashankara et al., 2013; Vasquez-Garzon et al., 2013), ginger treatment significantly ameliorated the elevation in AST at high dose level while no significant reduction in ALT activity was reported.

Oxidative stress and nitrosative stress, through generation of reactive oxygen species (ROS) and reactive nitrogenous species, act as an important influencing factor to carcinogenesis and both are substantial key factors in cancer as an end-stage of chronic diseases (Kawanishi et al., 2006). Current results demonstrated significant
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Alpha-fetoprotein (AFP) is a well-known tumor marker indicator of HCC (Sell et al., 1985). Being a growth regulatory cell-signaling factor, it has been reported to promote cell proliferation, suppress apoptosis, and act as an immunosuppressive agent (Mizejewski, 2014). High mortality and morbidity rates were reported to patients with AFP-positive-gastrointestinal cancer (AFP(+)/GC) than AFP(-)/GC-patients due to active cell proliferation, high mitotic rate, amplified cell invasion and migration, rapid tumor progression and advanced tumor stage (He et al., 2016).

Elevated serum levels of AFP have been detected in animals bearing liver tumors after treatment with certain hepatocarcinogens including DENA (Kroes et al., 1975). The increased level of AFP observed in DENA-induced animals is indicative of HCC (Jagan et al., 2008). In the present study, DENA induced significant elevation of AFP in liver, stomach and colon by 92, 82 and 86%, respectively, compared to normal groups. DENA-induced elevation in AFP was reported earlier (Das et al., 2016).

It was observed that stimulation of the nuclear oncogenes (c-fos, c-jun, c-myc) and the two gene transcripts of the AFP gene are triggered after 4-12 hrs and after 4-24 hrs, respectively, following turpentine-induced acute inflammation in the rat (Koj et al., 1983). Addition of AFP to skin cultures of human keratinocytes with

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T-lymphocytes resulted in boosted baseline expression of cytokines, chemokines, and growth factors (Potapovich et al., 2009). AFP exhibits a vital role in the regulation of tumor growth, cell differentiation and proliferation of human hepatoma cells through the AFP receptors (Li et al., 2002). Accordingly, elevated levels of inflammatory cytokines and NOx along with AFP, following DENA administration, support the notion that AFP serves as both an acute and a chronic phase reactant depending on its stage of ontogeny (Mizejewski, 2015). The majority of AFP-producing cancers originate from the stomach, bile duct, and pancreas. Clinically, eleven cases of colorectal cancer with only one case with early rectal cancer have been diagnosed as an AFP-producing tumor by immunohistochemistry (Anzai et al., 2015). Hepatic, gastric and colonic contents of AFP were markedly decreased by ginger extract pretreatment by approximately 50% compared to DENA group in a dose dependent manner. The chemopreventive activity of ginger extract and its constituents has been reported previously against myriad models of liver cancer (Mansour et al., 2010; Taha et al., 2010), gastric cancer rat models (Ko and Leung, 2010; Prasad and Tyagi, 2015b) and experimental colon carcinogenesis (Yoshimi et al., 1992; Manju and Nalini, 2006). Clinically, two grams daily of ginger supplement, to patients with increased risk for colorectal cancer, reduce proliferation in the crypts of normal-appearing colorectal epithelium and increase apoptosis and differentiation relative to proliferation (Citronberg et al., 2013).

The redox-sensitive transcription factor, nuclear factor-erythroid 2–related factor 2 (Nrf2), plays a central role in the inducible expression of genes encoding detoxifying systems, including phase II drug-metabolizing enzymes; NADPH, NAD(P)H quinone oxidoreductase 1, glutathione peroxidase, ferritin, heme oxygenase-1 (HO-1) (Jaiswal, 2004). These defense enzymes are coordinately induced through the antioxidant responsive element (ARE) and are tightly regulated by Nrf2 (Nguyen et al., 2003). The attenuated expression of these enzymes in Nrf2-deficient mice has verified the role of Nrf2 in the regulation of many detoxifying and antioxidant enzymes under oxidative stress conditions; rendering Nrf2-deficient mice more vulnerable to carcinogen-induced toxicity and carcinogenesis (Enomoto et al., 2001; Ramos-Gomez et al., 2001). Diethyltrinitroamine administration significantly decreased hepatic, gastric and colonic Nrf2 by 78, 83 and 82%, respectively, after 7 days of administration. This result was reflected by the dramatic decrease in reduced GSH content of the investigated tissues besides the increase in oxidative stress marker (MDA) and inflammatory markers (IL-1β and TNF-α). Similarly, a recent study reported that DENA down-regulates Nrf2 in the liver along with induction of oxidative stress, inflammation and angiogenesis (Mahmoud et al., 2017). Both doses of ginger extract protected liver, stomach and colon from DENA-induced decrease in Nrf2 with significant difference between low and high dose of GE. Previous studies showed increased antioxidant enzymes including GSH, SOD, and GPx by GE (Jeena et al., 2013). Zerumbone, component of Asian ginger oil, elevates phase II detoxification enzymes as well as nuclear localization of Nrf2/ARE (Nakamura et al., 2004). The upregulation of Nrf2 by ginger extract could exert an anti-inflammatory effect through elevation of HO-1 expression leading to the inhibition of NFκB signaling (Chi et al., 2015) giving a new insight in cancer prevention through upregulation of Nrf2/ARE pathway by ginger consumption.

Ginger inhibits transcription factor NF-κB, inflammatory cytokine TNF-α and targets several cellular molecules that contribute to tumorigenesis, cell survival, cell proliferation, invasion, and angiogenesis in different forms of GI cancers. Those molecular targets of ginger indicate that it may have the potential for preventing and treating GI cancer (Prasad and Tyagi, 2015b). Though the notion that Nrf2 inducers and/or Keap-1 suppressors may serve as promoters of cancer cell proliferation with increased resistance to ferroptosis cell death (Fan et al., 2017); ginger extract exerted an Nrf-2-inducing activity with concurrent inhibition of alpha-feto protein, proliferation marker, in all examined tissues and decline in oxidative and inflammatory markers, thus contributing to its chemoprevention activity probably via mechanism involving Nrf2/Keap1/ARE pathway. Therefore, further molecular investigation is warranted to outline ginger antioxidant/anti-inflammatory/anti-proliferative crosstalk mechanism.

Taking together current observation and previous supporting literature, GE supplementation ameliorated the distortion in liver architecture induced by DENA through hepatoprotective; antioxidative, anti-inflammatory, anti-proliferative and chemopreventive properties as evident by current histopathologic examination of liver tissues.

In conclusion, Ginger Extract alleviated DENA-induced decrease in reduced GSH, increase in MDA and NOx, elevations of IL-1β, TNF-α, and hepatic COX-2 expression, increase in AFP and decrease in Nrf2 of liver, stomach and colon of male Wistar albino rats via antioxidative, anti-inflammatory, and eventually chemopreventive properties with proposed anti-proliferative effect by inhibition of AFP-producing tumor pathway.

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Conflict of Interests

The authors declare none.

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