Mohamed F. Ismail  
Doaa A. Ali  
Heba A. Badr

ULTRASTRUCTURAL STUDY ON THE PROTECTIVE EFFECT OF GINGER AGAINST THE TOXICITY OF 7, 12 DIMETHYLBENZ [A] ANTHRACENE (DMBA) ON THE LIVER OF ALBINO RATS

ABSTRACT:
The present work was planned to investigate the protective effect of ginger against the ultrastructural changes induced by DMBA in the liver of female albino rats. 7,12dimethylbenz[a]anthracene (DMBA) is one of the strongest pollutants in soil, water and air. It induces neoplasms in the liver, heart and lungs. Animals were intraperitoneally given a single dose of DMBA (40 mg/kg body weight). After five months of treatment, the nuclei of the hepatic cells showed chromatinolysis and pyknosis. Hydropic degeneration of the hepatic cells and the breakdown of the mitochondria were also seen. The space of Disse and the bile canaliculi were very narrow and lost their microvilli. Oral administration of ginger (120 mg/ml) every other day simultaneously with DMBA for five months reduced the lesions that induced by DMBA. The obtained results suggest that the protective effect of ginger is mediated through the decrease of oxidation of lipids and the enhancement of antioxidants defences, thus minimizing the carcinogenic risk of DMBA.

INTRODUCTION:
Natural dietary agents including fruits, vegetables, and spices have drawn a great deal of attention from both the scientific community and the public due to their various health promoting effects, including suppression of cancers. Ginger is an example of botanicals that are gaining popularity amongst modern physicians (Gilani and Rahman, 2005). A considerable number of researches were carried out on ginger and its pungent constituents and fresh and dried rhizome. Some phenolic substances present in ginger generally possess a strong anti-inflammatory, anti-hepatotoxicity, and antioxidative properties and exert substainal anti-carcinogenic and anti-mutagenic activities. These agents are believed to suppress the transformative, hyperproliferative and inflammatory processes that initiate carcinogenesis as well as angiogenesis and metastasis (Gujral et al., 1978; Sambaih and Srinivasan, 1991; Park et al., 1998; Surh et al., 1999; Surh, 2002).

The compound 7, 12 dimethylbenz[a]anthracene (DMBA) is one of polycyclic aromatic hydrocarbons that constitute a class of ubiquitous environmental contaminants, primarily as the result of the occurrence in soil, coal-tar, petroleum and air pollutants (Cook and Dennis, 1988). DMBA induces neoplasms in the liver, heart and lungs after being metabolically activated (Dipple and Bigger, 1991). Moreover, DMBA causes elevation of lipid peroxidation in the liver (Takada et al., 1992). DMBA causes an increased generation of superoxide anions. This could be accompanied by a subsequent occurrence of free radicals corresponding to heightened lipid peroxidation coupled with a reduction of glutathione peroxidase (GSH-Px) activity (Chidamraram and Barodarajan, 1994).

The present work was conducted to study the hepatoprotective effect of ginger on DMBA-induced liver toxicity.

KEY WORDS:
DMBA, Ginger, Liver, Ultrastructure.

CORRESPONDANCE:
Mohamed F. Ismail  
Department of Zoology, Faculty of Science, Mansoura University, Mansoura, Egypt  
E-mail: heba_badr@mans.edu.eg

ARTICLE CODE: 24.01.09
MATERIAL AND METHODS:

Materials:

Ginger: the rhizomes of *Zingiber officinale* were brought from Metro market in El-Gomhorea Street, Mansoura, Egypt. They were shade dried at room temperature and were crushed to powder. 125 g of this powder were then macerated in 200 ml of distilled water for 12 hours at room temperature and were then filtered to obtain the final aqueous extract (120 mg/ml) as previously described (Kamtchouing *et al.*, 2002).

The carcinogenic material: 7,12-dimethylbenz[a]anthracene (DMBA) was purchased from Sigma (St. Louis, USA) and dissolved in corn oil.

Animals:

Healthy adult female albino rats (*Rattus rattus*), weighing about 55±5g were obtained from Helwan breeding farm and acclimated to the laboratory condition. Animals were fed on a standard diet and given water *ad libitum* for five months.

Treatment protocol:

The animals were divided into four groups: Control group, animals (n=10 rats) were intraperitoneally injected with a single dose of 1 ml corn oil/kg body weight. Ginger treated group, animals (n=10 rats) were orally administrated 1ml (120 mg/ml) of the final aqueous extract of ginger every other day for five months. DMBA treated group, animals (n=10 rats) were intraperitoneally injected with a single dose of 40 mg DMBA/kg body weight as previously described (Macejova and Borko, 2001). DMBA and ginger treated group, animals (n=10 rats) were intraperitoneally injected with a single dose of 40 mg DMBA/kg body weight and were orally given 1ml of the final aqueous extract of ginger every other day for five months. The animals were sacrificed at the end of the experiment (i.e after five months) and the liver was immediately dissected out and fixed for two hours in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.2) at 4°C and post-fixed in 1% cold osmium tetroxide in 0.1M sodium cacodylate at pH 7.2 for three hours. The specimens were then dehydrated in graded ethanol and embedded in Epson-Araldite resin. Ultrathin sections were stained by uranyl acetate followed by lead citrate as described by Reynolds (1963) and examined on Joel Electron Microscope (JAPAN) operating at 60kV.

RESULTS:

A- Control animals:

In electron microscope preparations, the nucleus of the hepatocytes was large, spherical and centrally located. The nucleus is found to contain a large amount of electron-lucent euchromatin and scattered heterochromatin (Fig. 1).

![Fig. 1. Electron micrograph of hepatocyte in control rat showing the presence of central spherical nucleus (N), numerous mitochondria (M) and rough endoplasmic reticulum (RER) (X 7,500).](image1)

The cytoplasm of the hepatic cells from control animals showed a granular appearance. There are numerous rounded and elongated mitochondrial profiles with electron-dense matrix. Also, there are profiles of rough endoplasmic reticulum between the mitochondria (Figures 1-3). The rough endoplasmic reticulum showed ribosomes, which can be seen free in the cytosol. Some profiles of SER with dilated cisternae can be seen especially in the periphery of hepatocyte (Fig. 2).

![Fig. 2. Electron micrograph of adjacent hepatic cell borders in control rat showing zonula occludents (ZO), zonula adherens (ZA), bile canalicular (BC) and microvilli (MV)). Note the presence of mitochondria (M), rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER) in the cytoplasm of hepatocytes (X 10,000). Bile canaliculi showed wide channels formed of opposing hepatic cells. Microvilli of the hepatic cells are found to project into the lumen of the bile canalculus, and junctional complex secured the attachment of the hepatic cells around these canaliculi (Fig. 2). The plasma membrane of the hepatic cells facing the blood sinusoids is formed of microvilli. The hepatic sinusoids are extremely thin-walled with only one discontinuous layer of lining cells. The lining cells are consisted of endothelial cells and Kupffer cells (Fig. 3).](image2)
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ISSN: 2090 - 0511 On Line ISSN: 2090 - 0503 http://www.egyptseb.org

Fig. 3. Electron micrograph of blood sinusoid showing wide space of Disse (DI), numerous microvilli (MV), endothelial cell (EN) and Kupffer cell (KC). Note the presence of mitochondria (M), rough endoplasmic reticulum (RER) and part of the nucleus (N) in the adjacent hepatocytes (X 7,500).

B- Ginger treated animals:

The structure of the hepatic cells, the bile canaliculi and blood sinusoids were found to be quite normal as those of the control groups (Figs 4-6).

Fig. 4. Electron micrograph of hepatocyte in ginger-treated (120mg/ml) rat showing the normal appearance of the nucleus (N), mitochondria (M) and rough endoplasmic reticulum (RER) (X 7,500).

Fig. 5. Electron micrograph of hepatic cell in ginger-treated (120mg/ml) rat showing mitochondria (M), rough endoplasmic reticulum (RER), zonula occludent (ZO), microvilli (MV) and bile canaliculus (BC) (X 13,000).

C- DMBA-treated animals:

Like many other carcinogens, DMBA is also cytotoxic for the liver. Most of the nuclei showed distinct features of chromatinolysis as shown in figures 8&9, where chromatin material was found to be lost. In other foci, pyknotic nuclei can also be seen (Fig. 10).

Fig. 8. Electron micrograph of hepatocyte in DMBA-treated (40 mg/kg) rat showing large round nuclei with lucent euchromatin (N), nucleoli (NU), destructed mitochondria (M), extensive rough endoplasmic reticulum (RER) and wide intercellular spaces (*) (X 3,000).

Fig. 9. Electron micrograph of hepatic cell in DMBA-treated (40 mg/kg) rat showing part of nucleus (N), nucleolus (NU), destructed mitochondria (M), dilated rough endoplasmic reticulum (RER) wrapped the altered mitochondria (M) (X 10,000).

Fig. 10. Electron micrograph of hepatocyte in DMBA-treated (40 mg/kg) rat showing the altered mitochondria (M), extensive profiles of rough endoplasmic reticulum (RER) and pyknotic nucleus (PN) (X 7,500).

One of the most pathological changes was the hydropic degeneration which affected hepatocytes all over the liver. The hepatocytes became swollen and lost their cytoplasmic density (Figs 8-14). Due to the increase of the hepatic cell size, the blood sinusoids were affected, the cell junctions were rarely found, and the space of Disse lost its microvilli. Moreover, the bile canaliculi
were also very narrow and lost their microvilli (Figs 11, 13 & 14).

Fig. 11. Electron micrograph of hepatocyte in DMBA-treated (40 mg/kg) rat showing the destroyed mitochondria (M), extensive profiles of rough endoplasmic reticulum (RER), large lipid droplets (LD), very narrow space of Disse (DI) and activated Kupffer cells (KC) (X 7,500).

Fig. 12. Electron micrograph of adjacent hepatic cell borders in DMBA-treated (40 mg/kg) rat showing very narrow bile canaliculus (BC) without microvilli (X 7,500).

Figs 13&14. Electron micrograph of hepatic sinusoids in DMBA-treated rat showing activated Kupffer cells (KC) very narrow space of Disse (DI) (X 10,000 & 5,000, respectively).

One of the most interesting changes was the breakdown of the intermitochondrial limiting membrane with its cristae. Also the mitochondrial matrices lost their electron-density. Other mitochondria were found completely broken down and their internal granular material discharged into the cytosol. The latter tiny flocculent materials give the cytoplasm its granular appearance. Representative electron-micrographs of hepatic cells showed a close association between mitochondria and RER. Profiles of RER were found wrapping the mitochondria in latter electron micrographs (Figs 9-11).

Moreover, large fat droplets were obviously seen in most cells. Other cell organoids were sparsely found may be due to the hydropic degeneration. The vacuolated endothelial cells and the active Kupffer cells were also found (Figs 11, 13 & 14).

D- DMBA and Ginger-treated animals:

The ultrastructural micrographs showed improvements in the DBMA induced changes in the hepatic cells. The nuclei were found to be more or less similar to those of control groups (Fig. 15). The mitochondria in most instances were found to be normal with electron-dense matrices; in the other hepatocytes the matrix was still electron-lucent. The rough and smooth endoplasmic reticulums were found to be similar to those of control profiles (Figs 15-18). Additionally, hydropic degeneration was still persisted in some hepatocytes. The bile canaliculi were increased in size as those of the control groups. However, their microvilli were not formed and the tight junctions between bile canaliculi and intercellular spaces were still incompletely formed (Fig. 17).

Fig. 15. Electron micrograph of hepatic cell in DMBA- and ginger-treated rat showing normal appearance of the nucleus (N), electron-lucent matrix of mitochondria (M) and normal rough endoplasmic reticulum (RER) (X 7,500).

Fig. 16. Electron micrograph of hepatic cell in DMBA- and ginger-treated rat showing normal appearance of mitochondria (M) and profiles of rough endoplasmic reticulum (RER), and hydropic degeneration (X 10,000).
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DISCUSSION

7,12 dimethylbenza[a]anthracene (DMBA) is a potent carcinogen and one of the polycyclic aromatic hydrocarbons. They are found throughout the environment in the air, water, and soil. They can occur in air, either attached to dust particles or as solids in soil or sediments (Windholz, 1983). Exposure to multiple doses of carcinogenic agent like DMBA directly or indirectly causes dose-dependent DNA damage and exposure to stress that predispose the individual to an increased risk of developing cancer (Muqbil et al., 2006).

On the other hand, the blood sinusoids had the characteristic appearance of those of control counterpart. However, in other instances these structures had the characteristic features of the DMBA-treated animals. The space of Disse was still unformed, the endothelial cells were found to contain numerous vacuoles and the Kupffer cells were still large and active (Figs 18&19).

The present results showed chromatinolysis in most of the nuclei, the cell junctions were rarely found and the space of Disse lost its microvilli and the bile canaliculi were very narrow and lost their microvilli. Also, breakdown mitochondria and large fat droplets were obviously seen in most cells. These results may reflect the carcinogenicity or toxicity of DMBA (or both) to the liver tissue. However, they may be also attributed to the increased production of reactive oxygen species and inhibition of antioxidant enzymes, or to disturbance of their production.

The present work is in agreement with Goldberg (1980) who reported that GGT is elevated in liver diseases and its increased activity in serum can occur as a result of the toxic effect of drugs on microsomal structure in liver cells and with that of Farag-Alla and Abdel-Dayem (2001) who attributed the elevation of GGT to the lytic effect of DMBA on the liver cytomembrane. This is shown in the present investigation by chromatinolysis and degenerative effects of DMBA on the hepatocyte.

Lesko et al. (1982) and Chidambaram and Barodarajan (1994) found that DMBA produces a much higher concentration of free radicals than do non-carcinogenic compounds. It is well documented that free radicals can damage DNA, structural proteins, enzymes, membranes and lead to toxic product formation (Hollander et al., 2001).

The strongest carcinogen, DMBA and the non-arguably weakly carcinogen, anthracene, was found to be readily transformed to radical cation which underwent reaction with chloride anion of water. Thus, these radical cations, if formed biologically in the nucleus, could react with nucleophilic sites on DNA as a possible mechanism to account for their carcinogenic potency (Chen et al., 1996).

On the other hand, the protective effect of ginger concomitant with DMBA treatment of the present study is represented by improvement of most ultrastructural alterations in liver tissue induced by DMBA.

The present observations reinforce the view that ginger was scavenging free radicals produced by DMBA through its potent antioxidant property. The present work is in agreement with Siddaraju and Dharmesh (2007) who reported that ginger free phenolic (GRFP) and ginger hydrolysed phenolic...
(GRHP) fractions of ginger (Zingiber officinale) exhibited free radical scavenging, inhibiting of lipid peroxidation, DNA protein and strong antioxidant properties.

Moreover, Toda et al. (1985), Kikusaki and Nakatani (1993), and Aeschbach et al. (1994) reported that chemical constituents like gingerol, shogaols, curcumin and zingerone present in ginger exhibited a strong antioxidative property. Pungent ginger ingredients such as 6-gingerol and 6-paradol have shown in vitro an anti-tumor activity against different cell lines (Keum et al., 2002).

The major pungent constituent of ginger, [6]-gingerol has been reported to exhibit antioxidative activity against linoleic acid autoxidation and peroxidation of phospholipid liposomes and to scavange trichloromethylperoxyl- and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals (Aeschbach et al., 1994; Sekiwa et al., 2000). In addition to these antioxidative effects, Ippoushi et al. (2003&2005) revealed that [6]-gingerol inhibits nitric oxide synthesis in activated macrophages and prevents oxidation and nitration reactions induced by peroxynitrite, a strong reactive nitrogen species (Radi et al., 2001). Besides [6]-gingerol, ginger contains a homologous series of phenolic ketones expected to have an antioxidative effect, known as [4]-, [8]-, [10]- and [12]-gingerols (He et al., 1998).

In the present work, ginger did not cause any side effect or organ toxicity. This is in accordance with Sakamoura and Hayashi (1978), Smith and Robison (1981), Nishimuro (1995), and Bartly and Jacobs (2000).

In conclusion, ginger may significantly reduce the potential of abnormal toxic and preneoplastic lesion induced by DMBA.

REFERENCES:


