

Protective Role of Lycopene Against Diethylnitrosamine Induced Experimental Hepatocarcinogenesis

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Abstract

Lycopene was considered as a major carotenoid in the human diet for only the last few centuries. Recently lycopene has been found to possess chemoprotective effect against gastrointestinal tract, urinary bladder, prostate and breast cancers. In the present study, the protective effect of lycopene, the natural extract from tomato pomace against diethylnitrosamine (DEN)-induced hepatocellular carcinoma (HCC) formation in rats was investigated. Four groups of male albino rats at the age of 6 weeks were studied: 1) control (C), 2) DEN, i.p. injected 5 times with doses of 200 mg/kg b.w., one dose every two days. 3) Lycopene, orally given 0.12 mg/rat/day. 4) Lycopene + DEN, the treatment with lycopene started 7 days before DEN injection and continued till the end of the experimental period. Rats in group 2 and 4 were treated with Phenobarbital (PB) at a dose of 500 ppm in the drinking water as a tumor promoter. PB treatment started with the first injection of DEN and continued till the end of the experimental period. The results indicated that DEN caused HCC nodules as evidenced by a remarkable significant increase in α -L-fucosidase and metalloproteinases (MMPs) enzyme activity in both serum and cytosol. Increased activity of these enzymes is a marker for both preneoplastic and carcinoma lesions. Administration of lycopene prior to DEN injection protected rats from DEN-induced HCC as evidenced by a significant descent in α -L-fucosidase and MMPs enzymes activity in both serum and cytosol. The histological investigation of liver tissue confirmed these results. DEN group showed proliferated hyperplastic and anaplastic hepatocellular nodules surrounded with thin layer of fibroblastic cells, desmoplasia and inflammatory cells infiltrations, focal hepatic haemorrhages and necrosis. Meanwhile, lycopene + DEN group showed no histopathological changes. These results indicate that lycopene effectively inhibits DEN-induced hepatocarcinogenesis in rats.

Introduction:

Diethylnitrosamine (DEN) belongs to the relatively small group of carcinogens that has been thoroughly investigated. It induced hepatocellular carcinomas, which is one of the most common endpoints in cancer tests (Finnberg *et al.*, 1999). DEN is considered to be a genotoxic carcinogen (Lewis *et al.*, 1997). Since it is positive in tests for genotoxicity and can induce many types of DNA damage and alterations that lead to mutations by alkylation and other mechanisms (Nakae *et al.*, 1997). Phenobarbital (PB) is a non-genotoxic carcinogen and has often been used as a tumor promoter (Osanai *et al.*, 1997).

The presence of nitrosamines in foods is considered a risk factor because of their

implication in causing gastrointestinal cancers of the pharynx, oesophagus, stomach, liver, pancreas and colorectum (Chhabra *et al.*, 1996, Jen and Yuan, 1994 and Magee, 1996). Since nitrosamines are suspected carcinogens in humans because the intestinal bacterial flora can transform nitrites and nitrates in the food into powerful carcinogenic nitrosamines (Ames and Gold, 1990), the mechanisms of their toxic action have been the focus of attention for several years (Lin and Ho, 1994 and Yang and Smith, 1996).

Hepatocellular carcinoma (HCC) is one of the major malignant tumors in humans and causes more than 250,000 deaths annually worldwide (Kuo *et al.*, 1998). It is closely associated with hepatic

fibrosis or cirrhosis. Fibrosis arises from overproduction of extracellular matrix as a result of activation of hepatic nonparenchymal cells including kupffer cells and stellate cells (Pinzani *et al.*, 1998). Activation of hepatic stellate cells in the fibrotic liver causes morphological changes in myofibroblast like cells, which is accompanied by synthesis of large quantities of extracellular matrix (Freidman, 1993). Prognosis of cirrhosis is very poor because the diagnosis is generally late, when the disease is so advanced that any effective treatment is precluded. Therefore, early detection is important in the management of this type of cancer (Pateron *et al.*, 1994 and Tangkijvanich *et al.*, 1999).

Recently, serum activity of alpha-L-fucosidase enzyme has been used as a marker for early detection of HCC in cirrhotics (Giardina *et al.*, 1998). Alpha-L-fucosidase enzyme (Ec 3.2.1.51) is a lysosomal enzyme present in all mammalian cells. Deugnier (1984) showed that serum alpha-L-fucosidase activity was significantly increased in patients suffering from HCC when compared to healthy subjects.

Matrix metalloproteinases (MMPs) are a family of enzymes classified as zinc-dependent proteinases that collectively can degrade the various structural components of the extracellular matrix, their specific proteolytic targets have since expanded to many other extracellular proteins. These enzymes are involved in processes such as wound healing and also in tumor invasion and metastasis (Coussens and Werb, 1996 and Wood and Archer, 2001).

Lycopene has been a major carotenoid in the human diet for only the last few centuries. Tomatoes and tomato products account for the majority of lycopene ingestion (Clinton, 1998). Lycopene is a carotenoid present in human blood and tissues (Stahl and Sies., 1996). Rather high levels of lycopene was found in liver, adrenals, and testis, whereas other tissues such as lung and kidney contained less (Stahl *et al.*, 1992 and Tanumihardjo *et al.*, 1990). Absorption and distribution of lycopene into different tissues of rats was studied after application of radioactively labeled lycopene (Mathews-Roth *et al.*, 1990). Liver was the major organ for lycopene accumulation. Recent

interest in lycopene is due to the findings of an inverse association between dietary lycopene and risk for some types of cancer including those of oral cavity, pharynx, esophagus, stomach, rectum (Franceschi *et al.*, 1994), liver (Matushima-Nishiwaki *et al.*, 1995), colon (Franceschi *et al.*, 1994 and Narisawa *et al.*, 1998), urinary bladder (Helzlsouer *et al.*, 1989 and Okajima *et al.*, 1998), prostate (Giovannucci *et al.*, 1995) and breast (Zhang *et al.*, 1997).

The biological activities of lycopene include antioxidant activity, singlet oxygen quenching and peroxy radical scavenging (Stahl and Sies, 1996). Although lycopene has no provitamin A activity, it exhibits other biological properties such as suppression of cell proliferation of human cancer cells (Levy *et al.*, 1995) and induction of gap Junction communication (Zhang *et al.*, 1991).

The objective of this study was to evaluate the protective effect of lycopene, the natural extract from tomato pomace against hepatocellular carcinoma induced by DEN, a potent hepatocarcinogen.

Material and Methods:

Experimental animals:

Male Sprague Dawley rats, 5 weeks of age were obtained from the animal house of the National Research Centre, Dokki, Egypt. They were kept for 12 weeks under good ventilation and illumination conditions and allowed standard casein diet and water ad libitum. Rats were acclimatized for one week before experimental use.

Treatment:

DEN

DEN (C₄H₁₀NO₂) was purchased from Sigma Chemical Co. (St Louis, MO, USA). It was in the form of solution packaged in a 100 ml serum bottle with butyl rubber stopper and aluminum tear seal. The bottle content was dissolved in 100 ml saline to make a 1% solution. Tumor initiation was achieved by 5 times injection with DEN at a dose of 200mg/kg b.w., one dose every two days according to Ito *et al.* (1998).

PB:

PB was purchased from Pfizer Pharm. Ind. Co., Cairo, Egypt. Tumor promotion was induced by adding PB at a dose of 500 ppm in the drinking water according to (Finnberg *et al.*, 1999).

Extraction and purification of lycopene:

Lycopene was extracted from tomato processing wastes or pomace obtained from tomato sauce processing line in the factory of Kaha. Extraction with diethylether, concentration and purification of lycopene were performed using the method described by Kew and Berry (1970). The purity of lycopene reached 95% as assessed by high performance liquid chromatography (HPLC).

Experimental design:

The experimental animals were divided into four groups, each of ten rats as follows:

1. Control group of rats only given standard casein diet.
2. Group of rats i.p. injected 5 times with DEN at doses of 200 mg/kg b.w., one dose every two days.
3. Group of rats orally given 0.12 mg of lycopene daily according to Narisawa *et al.* (1996) till the end of the experimental period.
4. Group of rats orally given 0.12 mg of lycopene daily starting 7 days before DEN injection and still continued till the end of the experimental period.

Rats in group 2 and 4 were treated with PB (500 ppm in the drinking water) starting with the first injection of DEN and continued till the end of the experimental period. At the end of the treatment time, blood samples were collected after 12 h. from fasting rat groups using the orbital sinus technique, then centrifuged at 3000 r.p.m. for 10 minutes. The clear supernatant serum was frozen at 20°C for the subsequent analysis.

Extraction of cytosol from liver tissue:

Liver was dissected out, then 0.5 g liver tissue was homogenated in 10 ml of distilled water at 4°C. The homogenate was centrifuged for 20 minutes at 4°C at 12000 r.p.m. The supernatant was taken and recentrifuged for 30 min at 15000 r.p.m. at

4 °C. Collected sera and cytosol were used for the determination of α -L-Fucosidase and MMPs enzymes.

Biochemical methods:

α -L-Fucosidase enzyme:

Activity levels of α -L-fucosidase enzyme in serum and cytosol were assayed by the modification of the method according to Zielke *et al.*(1972). 10 μ L of serum were added to 50 μ L of the substrate mixture, p-nitropheny L- α -Fucopyranoside dissolved in phosphate buffer (PHS) and incubated for 1 hour at 37°C. The reaction was stopped by adding 3.5 ml of stop buffer pH 10.5 (115 mM sodium carbonate, 15 mM glycine and 7.5 mM sodium chloride). Blanks were prepared in the same way but the incubation step was omitted. Absorbance of p-nitrophenol was read at 400 nm. The change in absorbance of the liberated p-nitrophenol was directly proportional to the activity of α -L-fucosidase enzyme.

MMPs enzyme:

Gelatin zymography: The gelatinolytic activity of MMPs was evaluated according to the method described by (Nagata *et al.*, 1976). Serum and cytosol (40 μ g of total protein per lane) underwent electrophoresis 10% polyacrylamide gels containing 10% sodium dodecyl sulfate (SDS) and gelatin type B (1 mg/ml) under non-reducing and without boiling. After electrophoresis, the SDS was removed from the gels by washing with 2.5% Triton X-100 at room temperature, and the gels were incubated overnight at 37°C with gentle shaking in Tris 50 mM pH 7.5, containing CaCl₂ 10mM and ZnCl₂ 1mM in order to activate metalloproteinases substrate digestion. After staining with coomassie blue and destaining, the final gel had a uniformly blue background except in regions to which MMPs and collagenase (standard) have migrated and activated the development of lysis bands.

Densitometric Scans: Band intensity was quantified by means of densitometric scanning using a system incorporating a video camera and a computer analysis package, the results were calculated as μ g

collagenase for MMPs/40 µg total protein from serum and cytosol.

Histological study:

Specimens from liver were fixed immediately in 10% neutral buffered formalin, dehydrated in different grades of alcohol, cleared in xylol, embedded in paraffin wax, sectioned at 4-6µ thick and stained with Haematoxylin and Eosin (Bancroft *et al.*, 1996) and examined microscopically.

Statistical analysis:

The results were statistically evaluated using Student's "t" test (Snedecor and Cochran, 1989).

Results:

Table(1):Serum and cytosol α-L-fucosidase enzyme activity in different groups of animals (µ mol PNP/hr/L).

Experimental groups	Serum	Cytosol
Control	2.26 ±0.19 (100%)	2.63 ± 0.23 (100%)
DEN	2.82 ± 0.02 ^a (124.8 %)	3.19 ±0.09 ^a (121.3%)
Lycopene	2.19 ± 0.14 (97.0 %)	2.52 ± 0.13 (95.8%)
Lycopene + DEN	2.33 ± 0.17 (103.1 %)	2.74 ± 0.19 (104.2 %)

-Each value represents the mean of 10 rats ±SE.
 - * Significant difference from control at P<0.05 and ** highly significant difference at P < 0.01.
 - a: Significant difference between DEN and lycopene +DEN groups.

The activity levels of α-L-fucosidase enzyme in the serum and cytosol of rats receiving DEN and/or lycopene are presented in Table (1). Injection of DEN to rats induced significant increase in α-L-fucosidase enzyme activity in both serum and cytosol as compared with the control group, the percentage of increase from control equal to 124.8 and 121.3% in serum and cytosol respectively.

The intubation of lycopene induced slightly non-significant decrease in the enzyme levels comparing to the control group in both serum and cytosol. Administration of lycopene prior to DEN injection resulting in less pronounced changes than

those recorded in rats injected with DEN, there were non-significant changes between (Lycopene + DEN) and control groups in both serum and cytosol. Meanwhile, there were significant differences between (Lycopene + DEN) group as compared with the DEN group in both serum and cytosol.

Results in Table (2) showed measurement of MMPs activity levels in serum and cytosol in male albino rats, to investigate the preneoplastic lesions growth in liver induced by DEN. The MMPs activity was resolved into its active form (92 KDa) of bands that appeared in the gel Fig (1&2) for cytosol and serum, respectively. The results indicated that injection with DEN caused a marked significant elevation in MMPs enzyme activity in cytosol and highly significant difference in serum as compared with the control group. The percent changes of increase from control in DEN group were 145 and 135.4% in serum and cytosol respectively.

Table (2): Serum and cytosol MMPs enzyme activity in different groups of animals.

Experimental groups	Serum	Cytosol
Control	10.75 ±0.8 (100%)	5.17 ± 0.39 (100%)
DEN	15.59± 1.2 ^{**a} (145 %)	7.0 ± 0.5 ^a (135.4%)
Lycopene	12.26 ± 1.19 (114 %)	6.03 ± 0.74 (116.6%)
Lycopene + DEN	12.49 ± 1.08 (116.2 %)	5.48 ± 0.47 (105.9 %)

-MMPs expressed as µg collagenase /40 µg total protein.
 -Each value represents the mean of 10 rats ± SE
 - * Significant difference from control at P<0.05 and ** highly significant difference at P < 0.01.
 - a: Significant difference between DEN and lycopene + DEN groups.

Administration of lycopene caused non-significant changes in the enzyme activity comparing to the control group in both serum and cytosol. Ingestion of lycopene pre-DEN injection revealed significant amelioration effects, there were significant differences between DEN and lycopene + DEN groups in both serum and cytosol. Meanwhile, the changes between control and lycopene + DEN groups were non-significant in both serum and cytosol.

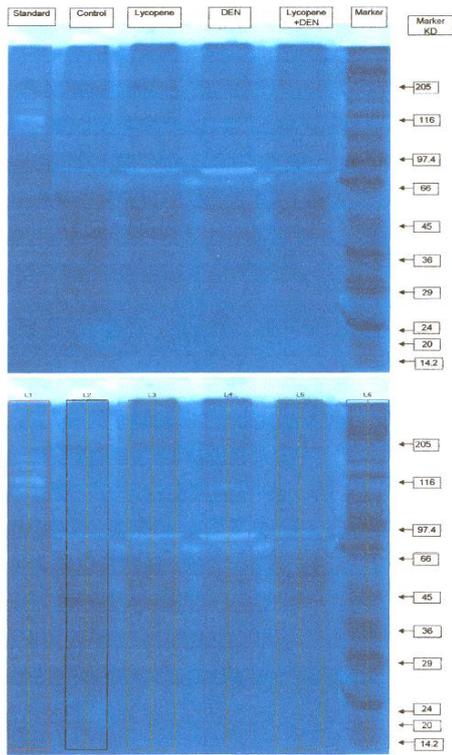


Fig (1): Gelatin zymography of SDS PAGE for rats liver MMPs activity levels

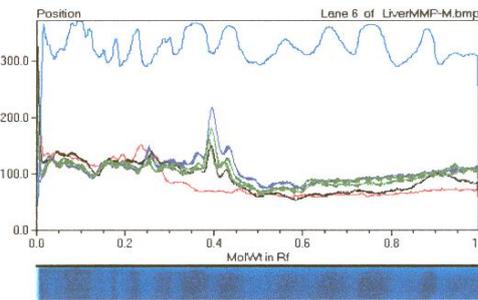
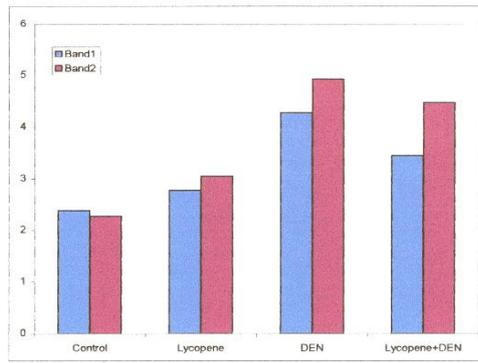


Fig (1): Densitometric scans of SDS PAGE for rats liver MMPs activity levels

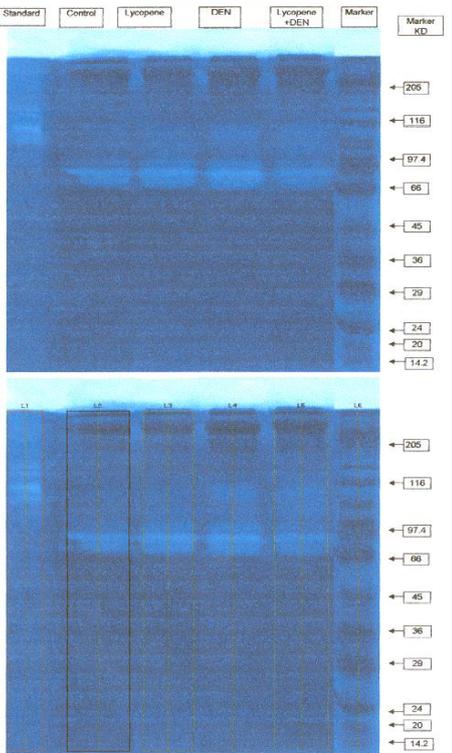


Fig (2): Gelatin zymography of SDS PAGE for rats serum MMPs activity levels

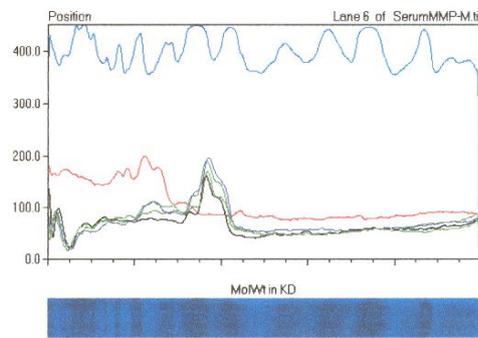
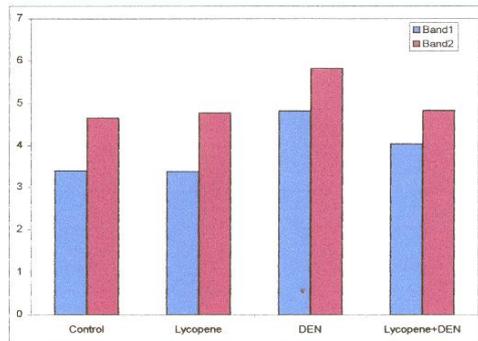


Fig (2): Densitometric scans of SDS PAGE for rats serum MMPs activity levels

Histopathological results:

Microscopical examination of liver from control rats showing no histological changes (Fig. 3). On the other hand, examined liver from rats treated with (DEN) revealed severe and deleterious changes, those changes confined as multiple hyperplastic and anaplastic hepatocellular nodules surrounded with thin layer of fibroplastic cells, absence of the central veins. The nodules made up of cells that resemble liver cells, but were anaplastic, haphazardly arranged and vary in their size and staining features (some clear cells with faint cytoplasm and other cells deeply stained) (Figs. 4 and 5). Mitosis and enlarged nuclei

were also seen in the examined cases . The portal tract showed irregular sized bile ductules, lined with cuboidal epithelium, which resembles epithelium of intrahepatic bile ducts, Less differentiated cells appear in solid masses with large nuclei and mitotic figures also seen. In some cases, proliferation of connective tissue (desmoplasia) and inflammatory cells infiltrations were also observed (Figs. 6 and 7).

Hepatic haemorrhages associated with necrosis were noticed in the examined sections (Fig. 8). Liver of rats treated with lycopene showed normal histological structure (Fig. 9). Liver of rats treated with combined lycopene + DEN also showed no histopathological changes (Figs. 10 and 11).

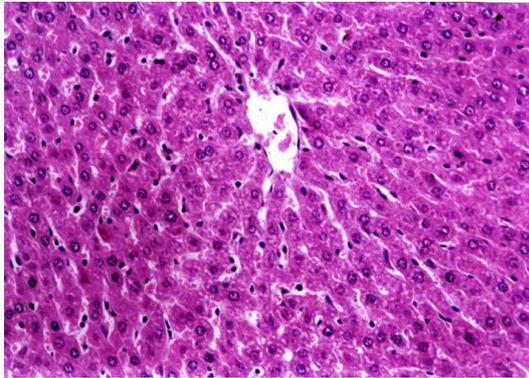


Fig. (3): Liver of control, non-treated rats showing no histological changes. (H &E stain X 200)

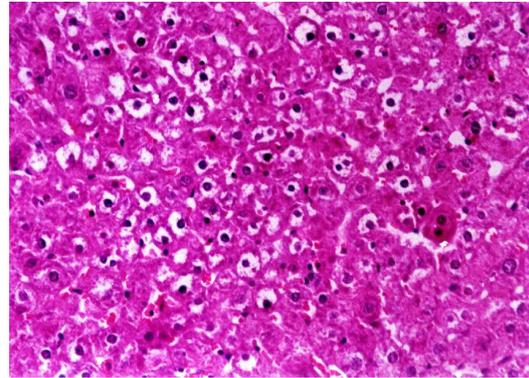


Fig. (4): Liver of rats treated with (DEN) showing proliferated hyperplastic hepatocellular and analplastic nodule (no central vein and the hepatocytes vary in their size and staining affinity) . (H & E stain X 200)

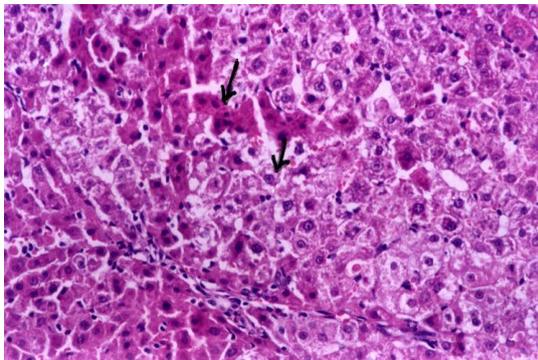


Fig. (5): Liver of rats treated with (DEN) showing mixed cell foci (clear cell hepatocytes and basophilic cell hepatocytes), notice the haphazardly arranged hepatocytes. (H & E stain X 200)

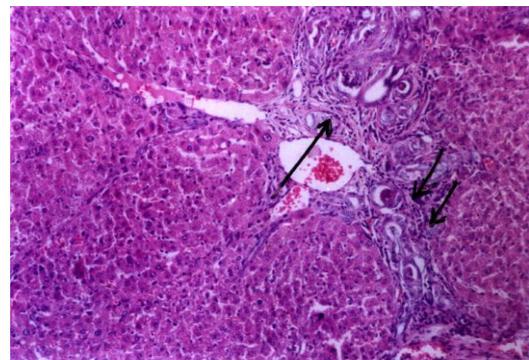


Fig. (6) : Liver of rats treated with (DEN) showing proliferated irregular sized bile ductules as well as desmoplasia and inflammatory cells infiltration (arrows) (H & E stain X 100)

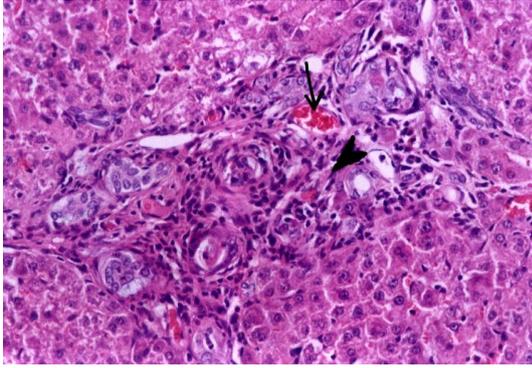


Fig. (7): Liver of rats treated with (DEN) showing proliferated bile ducts, the cells undifferentiated and form solid masses (arrow) as well as desmoplasia associated with inflammatory cells infiltration (arrow head). (H & E X 200)

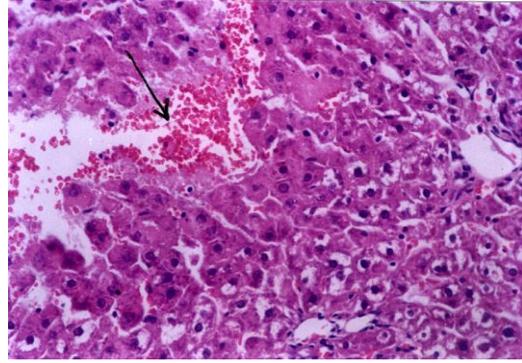


Fig. (8) : Liver of rats treated with (DEN) showing focal hepatic haemorrhages and necrosis (arrow). (H & E stain X 200)

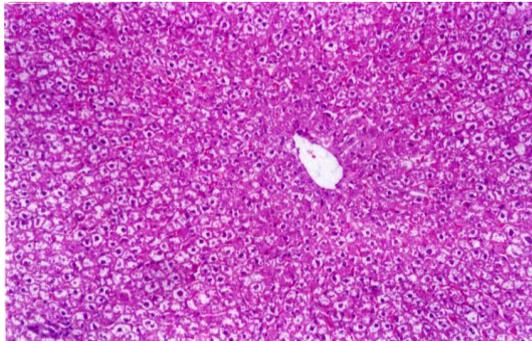


Fig. (9): Liver of rats treated with (Lycopene) showing normal histological structure. (H & E stain X 100).

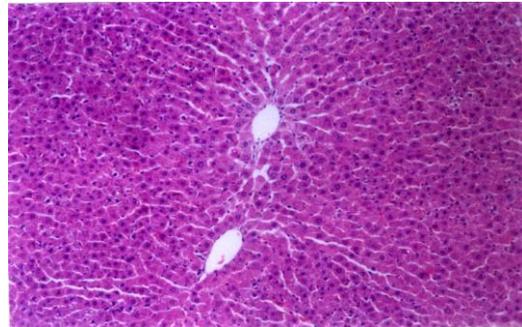


Fig. (10): Liver of rats treated with (Lycopene +DEN) showing no histopathological changes. (H & E X 100)

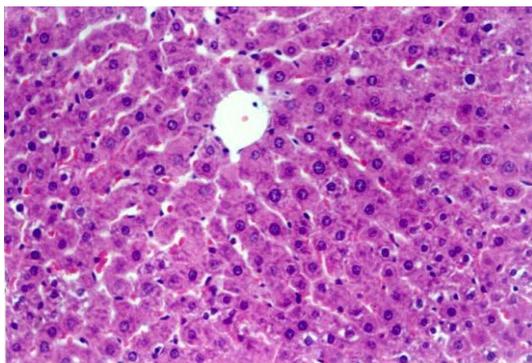


Fig. (11): Liver of rats treated with combined (Lycopene + DEN) showing no histopathological changes. (H & E stain X 200).

Discussion

It is well known that DEN is a potent hepatocarcinogen. Chuang *et al.* (2000) found that DEN induced HCC formation

in the mouse. The tumor initiation was achieved by a single i.p. injection of DEN (200 mg/kg b.w.). Nitrosamines are very common in processed foods such as bacon, dried and canned vegetables, beer and in cigar smoke. Humans are exposed to nitrosamines in several ways, and are especially effective by the oral route (Tricker and Preussmann, 1991). Rao and Agarwal (1999) reported the significant role of lycopene rich foods in prevention of chronic diseases and cancers in cell culture, animal experiments and clinics.

In the present study, DEN treated group showed a marked significant increase in α -L-fucosidase enzyme activity levels in both serum and cytosol as compared with the control group.

This finding was in agreement with the result of El-Houseini *et al.* (2001) who reported that in patients with HCC, α -L-fucosidase enzyme levels were significantly higher and correlated to the size of the tumor and the histopathologic grades. The increase in α -L-fucosidase may be attributed to increased synthesis of proteins by tumor with a consequent increase in fucose turnover (Deugnier, 1984). This finding is very important because it is well known that DEN confers hepatocarcinogenic action by its metabolic conversion to a reactive ethylating agent, which can alkylate cellular DNA (Bartsch *et al.*, 1989). DEN-activating P450 isozyme, markedly increased and may play a role in inflammation related tumor initiation. Inflammation also activates many proliferation factors and cytokines, which are involved in tumor promotion (Moshage, 1997).

The present study also revealed that group of rats administered lycopene prior to DEN injection showed a slight non-significant increase and significant elevation in α -L-fucosidase enzyme activity levels in both serum and cytosol as compared with the control and DEN groups, respectively. In vitro studies have shown a tumor suppressive activity of lycopene. Huang *et al.* (1997) stated that lycopene as antioxidant suppressed a severe hepatic inflammatory reaction induced by high dose of DEN in the rat. The mechanism for cancer preventing action of lycopene observed in animal models may involve antioxidant activity, stimulation of gap junction intercellular communication, induction of detoxifying enzymes, and inhibition of cellular proliferation (Cooper *et al.*, 1999).

The present results showed clear significant elevation in MMPs enzyme activity in both serum and cytosol of DEN group as compared with the control group. This was in agreement with Nakajima *et al.* (1993) who reported elevated level of MMPs in serum and plasma of animals bearing experimental tumors. Moreover, Moses *et al.* (1998) reported that elevated production of MMPs enzyme by cancer cells and tumor stroma correlates with the malignant or metastatic phenotype. Overproduction of MMPs by a tumor

communicating with the vascular and lymphatic systems might result in increased levels of MMPs activity in other body fluids, such as blood or urine.

On the other hand, MMPs play an important role in the tissue remodeling of the liver, these enzymes regulate the proliferation and migration of hepatocytes (Watanabe *et al.*, 2003). The early stage of angiogenesis is characterized by proteolytic degradation of basement membrane that involves active serines proteases and MMPs, a family of proteases with specificity for matrix molecules. It consists of collagenases, which cleaves collagen types I, II and III. These collagen fragments are further degraded by the gelatinases, 72 KDa gelatinase A (MMP-2) or 92 KDa gelatinase B (MMP-9) and by stromelysins (Nagase, 1996). The stage of tumor progression is positively correlated with the expression of MMP family members. Changes in MMP levels can markedly affect the invasive behavior of tumor cells and their ability to metastasize in experimental animal models (Coussens *et al.*, 2003).

In the obtained results, there was significant descent in MMPs enzyme activity in both serum and cytosol of lycopene + DEN group as compared with the DEN group. This finding was explained by Giovannucci (1999) and Mucci *et al.* (2001) who reported that lycopene may protect lipids, nucleic acids and proteins from oxidative damage that could otherwise lead to cancer. It is also possible that lycopene enhances cellular differentiation, inhibits cell proliferation, maintains intercellular communication, interacts with growth factors, or influences carcinogenesis through other mechanisms. Moreover, lycopene could influence the cancer cascade by regulating gene functions, modulating hormone status, carcinogen metabolism, immune status and angiogenesis (Folkman, 1995, Kobayashi *et al.*, 1996 and Agarwal and Rao, 2000).

The histological investigation of liver sections in rats injected with DEN revealed severe and deleterious changes varied from the presence of multiple hyperplastic and anaplastic nodules surrounded with thin layer of fibroblastic cells, desmoplasia and inflamm-

atory cells infiltrations, to focal hepatic haemorrhages and necrosis. These findings were in coincidence with the observations of Chuang *et al.* (2000) who found that DEN-induced hepatic nodules in rats fulfilled the histological criteria of HCC.

Moreover, Finnberg *et al.* (1999) reported that DEN treated rats developed enzyme altered foci (EAF) in rat liver and the longer the period of treatment the larger the size of the EAF. They found that many lesions in rats receiving DEN for 10-12 weeks had probably reached the point when conversion to a tumor was imminent. Deal *et al.* (1989) stated also that DEN may induce cytotoxicity and apoptosis. A progressive increase in replication of hepatocytes has been observed in response to increasing doses of DEN. It is a potent hepatocarcinogen, principally in the first step of neoplastic transformation (Cortinovis *et al.*, 1991). Lijinsky (1987) stated that initiation is the first damage suffered by the cell, if such damage is permanent it could lead to cancer. DEN has a great capacity for causing permanent genotoxic damage to cells, so it is an efficient cancer initiator.

On the other hand, DEN inducing cell death or a stop in the cell cycle in surrounding hepatocytes and by inducing adaptation to such effect, i.e., down regulation of P53 expression. The low levels of DEN may not down-regulate P53 expression. At high doses, it might become a dominating mechanism for accumulating mutations (Williams *et al.*, 1996).

Histological examination of liver sections of rats treated with lycopene confirmed the protective effect of lycopene in relation to liver structure. Concerning liver of rats treated with combined (Lycopene + DEN) showed no histopathological changes. This finding was coincided with the finding of Matushima-Nishiwaki *et al.* (1995). They recorded the effective role of lycopene in protecting against microcystin-LR-induced liver tumors in mouse hepatocytes. They suggested that lycopene may inhibit tumor promotion by suppressing the hyperphosphorylation of cellular proteins.

Furthermore, He *et al.* (1997) found that the intubation of rats with a carotenoid-rich extract from tomato paste decreased

the preneoplastic foci induced by aflatoxin during the initiation period, suggesting a beneficial role of lycopene in the early stages of hepatocarcinogenesis. Astorg *et al.* (1997) also found a decrease in the size of preneoplastic foci in rats liver as well as in the fraction of liver volume occupied after lycopene supplementation, but not after β -carotene, cantaxanthin and astaxanthin supplementation. This finding in the lycopene + DEN group may be attributed to the ability of lycopene to cause a reversible inhibition of cell transformation initiated by carcinogens. This activity was correlated with the ability of lycopene to upregulate gap junction communication by increasing the gene expression of connexin 43 (Zhang *et al.*, 1992), a component of the gap junction structure. Loss of gap junctional communication may be important for malignant transformation, and its restoration may reverse the malignant process.

In conclusion, the present data indicated the inhibitory effect of lycopene, the natural extract from tomato pomace on DEN induced hepatocarcinogenesis in rats which is likely to be related to the detoxification of the carcinogen through inhibition or by induction of enzymes. Alternatively, lycopene may have a role in interfering with carcinogen DEN-binding to elicit its antitumorigenic response. Otherwise, lycopene could play a role in repairing genetic lesions or by reinforcing immune surveillance mechanisms.

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الدور الوقائي للليكوبين ضد سرطان الكبد المستحدث بالداى إيثيل نيتروزأمين فى فئران التجارب

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يعتبر الليكوبين هو الكاروتين الرئيسى فى غذاء الإنسان خلال القرون القليلة الماضية فقط، و حديثاً أثبتت الدراسات التأثير الوقائى للليكوبين ضد سرطان الجهاز الهضمى، المثانة، البروستاتا و الثدي. وهذه الدراسة تهدف الى تقييم التأثير الوقائى للليكوبين، المستخلص الطبيعى من بقايا تصنيع الطماطم على تكون سرطان خلايا الكبد المحدث بواسطة الداى إيثيل نيتروزأمين فى الفئران. وقد إشمطت الدراسة على أربعة مجاميع من ذكور فئران الألبينو البالغة 6 أسابيع من العمر: (1)- المجموعة الضابطة، (2)- المجموعة التى حقنت 5 مرات تحت الغشاء المخاطى بالداى إيثيل نيتروزأمين (200مجم/كجم من وزن الفأر) جرعة واحدة كل يومين، (3)- المجموعة التى أعطيت الليكوبين بجرعة مقدارها (0.12 ملجم / فأر / يوم) و (4)- المجموعة التى أعطيت الليكوبين + الداى إيثيل نيتروزأمين بنفس الجرعات، تم إعطاء الليكوبين لمدة 7 أيام قبل الحقن بالداى إيثيل نيتروزأمين واستمر حتى نهاية التجربة. وقد تم إضافة الفينوباربيتول إلى مياه الشرب المقدمة إلى المجموعتين الثانية والرابعة كمادة محفزه للسرطان بداية منذ أول جرعة حقن بالداى إيثيل نيتروزأمين وحتى نهاية التجربة.

أوضحت النتائج أن الداى إيثيل نيتروزأمين أحدث تحفيز لتكونات سرطانية فى خلايا الكبد وقد ظهر ذلك بوضوح من خلال حدوث ارتفاع معنىى نو دلالة إحصائية فى نشاط أنزيمات الألفا-ل-فاكوسيديز و الماتريكس ميتالوبروتينيز فى كل من أنسجة الكبد و السيرم. يعد زيادة نشاط هذه الانزيمات أحد القياسات الدالة على حدوث كلا من التأثيرات السرطانية الضاره ، ومرحلة ما قبل ظهور النمو السرطانى . قد أدى إعطاء الليكوبين قبل الحقن بالداى إيثيل نيتروزأمين إلى وقاية الفئران من سرطان خلايا الكبد المحدث بواسطة الداى إيثيل نيتروزأمين ومن الدلائل على ذلك حدوث انخفاض معنىى نو دلالة إحصائية فى نشاط أنزيمات الالفال-فاكوسيديز و الماتريكس ميتالوبروتينيز فى كل من أنسجة الكبد و السيرم . وقد أكدت الدراسات الهستوباثولوجية لأنسجة الكبد هذه النتائج. حيث أوضح الفحص الميكروسكوبى لأنسجة الكبد زياده انقسام الخلايا وتكاثرها على نحو غير سوى، وجود عقيدات كثيرة تحيط بها طبقة رقيقة من الخلايا المكونة للالياف ، زيادة انقسام خلايا النسيج الضام، مع وجود التهابات و حدوث نزيف وموت موضعى لبعض الخلايا فى المجموعة المحقونة بالداى إيثيل نيتروزأمين ، أما المجموعة التى أعطيت الليكوبين قبل الحقن بالداى إيثيل نيتروزأمين وحتى نهاية التجربة فلم تظهر أى تغيرات با ثولوجية . من النتائج الحالية يتضح الدور الوقائى للليكوبين ضد سرطان الكبد المحدث بواسطة الداى إيثيل نيتروزأمين.