Original Communication

β-Carotene inhibits NF-κB and restrains diethylnitrosamine-induced hepatic inflammation in Wistar rats

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Abstract: β-Carotene exhibits antioxidant and hepatoprotective activities *via* a multitude of biochemical mechanisms. However, the action mechanism involved in antioxidant and anti-inflammatory effects of this carotene in chronic liver diseases is not fully understood. In the present investigation, we have attempted to outline a plausible mechanism of β-carotene action against liver fibrosis in albino Wistar rats. To induce hepatic fibrosis, diethylnitrosamine (DEN) was administered in experimental rats for two weeks. DEN treated rats were divided into four groups, wherein each group comprised of five rats. β-Carotene supplement attenuated DEN-induced elevation in LFT markers ($P < 0.05$); averted depletion of glycogen (24%, $P < 0.05$) and, increased nitrite ($P < 0.05$), hydroxyproline (∼67%, $P < 0.05$) and collagen levels (∼65%, $P < 0.05$). Confocal microscopy of tissue sections stained with picrosirius red revealed accrued collagen in DEN-administered group, which was found to be reduced by β-carotene supplementation. Furthermore, β-carotene decreased the expression of iNOS/NOS-2 and NF-κB, as revealed by immunohistochemistry and Western immunoblotting. Collectively, these results demonstrate that β-carotene mitigates experimental liver fibrosis *via* inhibition of iNOS and NF-κB *in-vivo*. Thus, β-carotene may be suggested as a possible nutraceutical to curb experimental liver fibrosis.

Keywords: β-Carotene, Hepatic fibrosis, iNOS, Diethylnitrosamine, NF-κB, Nutraceutical

Abbreviation

NF-κB = Nuclear Factor-κB, iNOS = Inducible Nitric Oxide Synthase, COX-2 = Cyclooxygenase-2, BOS = Board of Studies, CASR = Committee for Advanced Studies and Research, CPCSEA = Committee for the Purpose of Control and Supervision of Experiments on Animals, FCP = Folin and Ciocateu's Phenol, GnHCl = Guanidine Hydrochloride

Introduction

Carotenoids are fat-soluble organic pigments with mainly 40-carbon structure, found in many fruits and vegetables [1]. The two classes of these pigments are xanthophylls and carotenes. The xanthophylls are oxygen containing pigments (e.g. β-cryptoxanthin, zeaxanthin, lutein), while carotenes are oxygen devoid (e.g. β-carotene, α-carotene, lycopene) [1]. Although, carotenoids are extremely beneficial to human beings, their excessive consumption may produce toxicity [2]. β-Carotene, the most abundant carotene imparts orange color to many fruits and vegetables. This carotene is cleaved by lipoxygenase enzymes and also prevents oxidation of free radicals [3]. In humans, β-carotene is metabolized in the presence of enzymes mainly present in liver and adipose tissue other than the developing tissues such as placenta and yolk sac [4, 5]. These enzymes carry out the bioconversion of β-carotene to vitamin A, suggesting that β-carotene can serve as a source of retinoids in several sites within the body. It is further reported that β-carotene and its metabolites provide protection against light to photosynthetic plants, microorganisms, skin and eyes of animals. They are also known to prevent heart diseases, macular degeneration and cancer [6].

Liver is the most important vascular organ of the human body and it also plays a key role in metabolism and detoxification of various xenobiotics and drugs. Simultaneously, this organ is also susceptible to a wide range of toxic, metabolic, circulatory, microbial and neoplastic insults [7]. Chronic liver diseases including cirrhosis, non-alcoholic steatohepatitis (NASH), viral hepatitis and hepatocellular carcinomas are among the major concerns throughout the world. All of these chronic stages are known to pass through hepatic fibrosis which is the intermediate phase for all the above ailments. However, hepatic fibrosis is a reversible stage of liver damage. It is characterized by inflammation, collagen deposition and formation of scar tissue [8]. If fibrosis remains untreated, cirrhosis the irreversible stage of liver damage follows. Liver cirrhosis has been positively

correlated with the development of hepatocellular carcinoma (HCC), the terminal stage of liver damage [9, 10, 11]. Thus, to prevent the development of cirrhosis or carcinoma, it is imperative to restrain inflamed liver from entering fibrosis. DEN-induced acute liver injury in rodents is an established disease model to help discover novel therapeutics and their mechanism of action. The disease model perfectly resembles the mechanism of liver fibrosis in humans [12, 13, 14]. DEN undergoes metabolic activation in liver, producing active ethyl radical metabolites ($CH_3CH_2^+$) which initiate lipid peroxidation and cellular damage [15]. Literature also reveals that the intrinsic free oxyradicals play a crucial role in systematically initiating and modulating the progression of liver fibrosis [16, 17]. Reactive oxygen species (ROS) reportedly activates proinflammatory nuclear factor-κB (NF-κB). NF-κB regulates genes involved in the process of inflammation, cell survival, transcription and metastasis [18]. NF-κB is usually present in the cytoplasm, bound to its inhibitor IκB. Activation by ROS causes IκB derogation, which in turn, translocates NF-κB to the nucleus. This nuclear NF-κB then binds to DNA and induces the genes encoding pro-inflammatory proteins such as nitric oxide synthase-2/inducible nitric oxide synthase and cyclooxygenase-2 etc. [19]. Available reports also suggest that several powerful antioxidants and anti-inflammatory phytochemicals can suppress NF-κB signaling both *in-vivo* and *in-vitro* [20, 21].

β-Carotene has been found to protect against various hepatotoxins including nitrosamines [22, 23]. However, no data is available on the effect of β-carotene to regulate NF-κB during DEN-induced hepatic injury. In the present study, we have examined the potential of β-carotene in regulating NF-κB expression to regress DEN-induced liver injury in rodents.

Materials and methods

Chemicals

Chemicals procured from Sigma-Aldrich (USA) include diethylnitrosamine (DEN), β-Carotene, 2-Thiobarbituric acid (TBA), 2,4-Dinitrophenylhydrazine (DNPH), and Direct Red 80. *P*-Dimethylaminobenzaldehyde, sulphanilamide, *N*-(1-Naphthyl) ethylenediamine (NEDA) and Haematoxylin from SRL (India). NFκB p65 polyclonal antibodies (Rabbit) from Santa Cruz Biotech. iNOS and Goat anti-Rabbit IgG antibody, (H+L) HRP conjugate secondary antibodies from Puregene Genetix (India) and Sigma-Aldrich (USA) respectively. Immobilon western HRP substrate belong to Merck Millipore (India), while remaining chemicals or reagents and salts used in this study were of AR grade.

Animals

Albino rats (male) of 6–8 weeks age (Wistar strain) with an average body weight of 160 ± 10 g were used for the experimentation. Randomly picked animals were kept in polycarbonate cages under controlled conditions (25 ± 1 °C) in the animal house. Animals were divided into four groups with five (n = 5) animals each. These animals were given equal exposure of light-dark and had regular diet and drinking water *ad libitum*. The synopsis was approved by BOS and University CASR. Experiments were carried out in compliance with the CPCSEA, India.

Experimental design

The standard methodology of Matos et al. was followed to prepare β-carotene stock [24]. The dose of 20 mg kg^{-1} b. wt was prepared by adding normal saline drop by drop to the content followed by vortex for 3 min.

Healthy male rats were randomly selected and assigned to different experimental groups. These animals were grouped into four (G-1 to G-4) with five rats each. G-1, rats represented negative control (given 0.9% normal saline); G-2, animals received 10 mg/kg b wt of 1% DEN only once (prepared in normal saline); Rats in G-3 received a single dose of β-carotene (20 mg/kg b wt) per day for three consecutive days every week. G-3 was considered as positive control, and the β-carotene dose was ascertained based on LD50 (20 g/kg b wt) [25]; G-4, rats receiving DEN were also given β-carotene subsequently in the doses mentioned above for fourteen days. All of the doses were given intraperitoneally. The lag between doses of DEN and β-carotene in G-4 animals was kept to two hours. On the 15th day, the experimental animals were sacrificed by cervical dislocation. From the sacrificed animals, tissues were collected and processed for further analyses.

Preparation of sera and liver homogenates

Sera were obtained from the blood collected from the heart directly. Sera ooze out within 2 hours of keeping the tubes undisturbed at 25±1 °C. The ooze out sera (pale yellow in color) was collected in a separate microfuge tube avoiding contamination of RBCs. The mixture was centrifuged at ~1000g (10 min) to sediment cellular debris. The prepared sera were either examined afresh or stored properly (–20 °C) until use.

The dissected liver was washed in pre-chilled normal saline to remove blood or any other tissue debris and blotted dry to weigh. Liver tissue homogenate was prepared in pre-chilled alkaline buffer (1:3 w/v; Tris–HCl 50 mM, pH 7.5). The homogenates were centrifuged (8000 rpm, 30 min) at 4 °C. The resulting clear supernatants were stored for

liver biochemistry and Western immunoblotting analyses. A small quantity of liver was preserved in 10% formalin for histopathology and immunohistochemistry.

Protein estimation

To maintain accuracy in the biochemical estimates among different treatment groups of rats, protein estimation in the investigated tissues was performed by following the method of Lowry et al. [26]. The color in the reaction mixture was developed using Folin-Ciocalteu reagent. Following incubation of the reaction tubes, absorbance of the samples was determined at 660 nm. These values were compared with the known quantities of bovine serum albumin (BSA) standard.

Hepatic glycogen estimation

The glycogen levels in experimental animals were measured in liver [27]. Approximately 0.2g of liver was subjected to homogenization in 5% of trichloroacetic acid (20 mL) followed by low speed centrifugation (5000 rpm at 4 °C) for 20 min. KOH (10N) was added to the supernatant (1:1), and kept in boiling water bath for 60 min. The contents were brought to room temperature and 1 mL of glacial acetic acid was added to neutralize excess alkali in the solution. Approximately 2 mL of this mixture was poured in a glass tube placed in ice, and 4 mL of anthrone reagent was added. The contents were properly mixed and placed in boiling water bath for 10 min. Optical density of the color thus developed was measured at 650 nm and values were represented as mg/g tissue.

Hepatic protein carbonyls examination

To determine protein carbonyls in the animal liver, procedure of Levine et al. was used [28]. Briefly, tissue homogenate was mixed with 2,4-dinitrophenylhydrazine (1:4 v/v) prepared in 2.5 M HCl. Proteins present in homogenate were precipitated with 20% trichloroacetic acid. The collected pellet was washed three times with ethanol-ethyl acetate mixture (1:1) and centrifuged. The resulting pellet was solubilized in guanidine hydrochloride (GnHCl, 6 M) prepared in potassium phosphate (20 mM, pH 6.5). The absorbance was measured at 380 nm.

Nitrite estimation

Nitrite content in sera and liver of experimental rats was calculated as described previously [29]. Briefly, equal amount of sera and liver homogenate were mixed with Griess reagent (solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% NEDA in water, 1:1) and the reaction tubes were incubated (10 min) in dark at room temperature. A range of 10–100 mg of sodium nitrate ($NaNO_3$) was used to draw a standard curve. The optical density was recorded (540 nm) against the reagent blank.

Hydroxyproline and collagen content

Tissue hydroxyproline levels (liver) were estimated following the protocol of Woessner [30]. The tissues were incubated with 6N HCl for overnight in boiling bath. Acid traces were removed from the hydrolyzed specimens by keeping them in water bath. The obtained residue was dissolved in water and filtered. The filtrate mixed with fresh chloramine-T (1:1) was kept for 20 min and subsequently mixed with perchloric acid (3.15 mol L^{-1}). After 5 min, fresh 1 mL of p-dimethyl aminobenzaldehyde was mixed with constant shaking. The mixture was incubated in water bath (60 °C) for 20 min. Once samples attained the room temperature, the absorbance was read (560 nm). The levels of hydroxyproline were multiplied by a factor of 7.46 to obtain collagen content in the liver [31].

Direct red staining

For Direct red staining, the procedure of Junqueira et al. was followed [32]. Paraffin embedded ∼5μm thick liver sections were processed for the staining to confirm collagen accumulation and fibrosis. Properly stained sections were thoroughly examined under microscope and photographed at different magnifications (Nikon: 80*i*).

Immunohistochemistry of iNOS

Five μm thick tissue slices were deparaffinised with xylene, rehydrated with decreasing grades of ethanol and finally with water. Treatment with 3% H_2O_2 for 15 min blocked endogenous peroxidase activity in tissue sections. After a rinse in PBS, the sections were loaded with primary polyclonal NOS-2 antibody (dilution, 1:100) and incubated in wet chamber for overnight. After washing away unbound primary antibody in PBS, the sections were loaded with goat anti-mouse IgG secondary antibody (HRP-conjugated) for 2 hours. Subsequent to washing in PBS, the sections were incubated with DAB for 20 min. A final rinse in PBS was followed by counterstaining with Mayer's haematoxylin. Dried sections, mounted with DPX, were viewed under microscope.

Electrophoresis

Polypeptide profiling of liver was carried out in polyacrylamide gel electrophoresis, with minor modifications [33]. The electrophoretic runs (7%) were furnished (60 V, 5 mA /gel) in the presence of glycerol (10%) along with SDS in samples, gels and running buffers. Finally, the acrylamide gels were stained in CBBR250 as already described [34]. Molecular weight of the documented polypeptides was investigated by GelPro [35] against a broad range molecular weight marker (3.5–205 kDa).

Western blot analysis

Western blotting of iNOS and NF-κB was performed according to standard method of Sambrook et al. [36]. Polypeptides were electrophoretically resolved in 10% polyacrylamide gels (in the presence of SDS). The transfer of polypeptides was done into 0.45 mm thick PVDF membranes under constant conditions (125 V, 200 mA, 4 °C) for 2 hours. The membrane was subsequently washed thrice in 50 mM PBS (pH 7.1). Following incubation of membrane in non-fat milk (5% w/v in PBS) for 1 h, the membrane was then incubated with primary antibodies specific for NOS-2 and NF-κB (in dilution of 1:300 and 1:400, respectively) for 2 hours. The membrane was washed (PBS-T) thrice and treated with goat anti-mouse IgG (HRP-conjugated) secondary antibody (1:2000) for 1 hour. The antibody loaded membranes were washed (PBS-T) thrice to remove unbound secondary antibody and the immunoreactivity signals were documented using specific substrate. Scion Imaging and GelPro were run to find density of the selected blots.

Statistical analysis

The obtained results were presented as mean ± sem. Comparative analyses were carried out by ANOVA (one-way), and subsequently by Tukey's test (GraphPadInStat 3.1). Post-hoc Tukey's test determined the homogeneity of variance. The Excel NORMDIST was used to calculate normal distribution of the data. Values of the performed biochemical estimates were compared for their significant difference ($P < 0.05$, 0.01).

Results

Comparative phenotypic changes in livers of different groups indicated gross changes in DEN treated animals. The livers of these animals were yellowish in color with the characteristic presence of small nodules. Biochemical assays of serum AST, ALT, ALP and γGT exhibited remarkable increase in DEN administered animals within fourteen days (Figure 1a). In DEN-induced fibrotic animals, glycogen contents in the liver declined significantly (Figure 1b). β-Carotene supplementation in the prescribed doses significantly restored these changes ($P < 0.05$). Moreover, exposure to DEN resulted in the increase of hepatic protein carbonyl contents (~54%), signifying oxidative stress in the experimental animals ($P < 0.01$) (Figure 1c). The changes in protein carbonyls were also corroborated by the presence of nitrosative stress among fibrotic rats. The generation of nitrosative stress was further evident by significantly high levels of nitrites in the sera (~73%) and liver (~61%) of DEN-exposed animals (Figures 2a and 2b

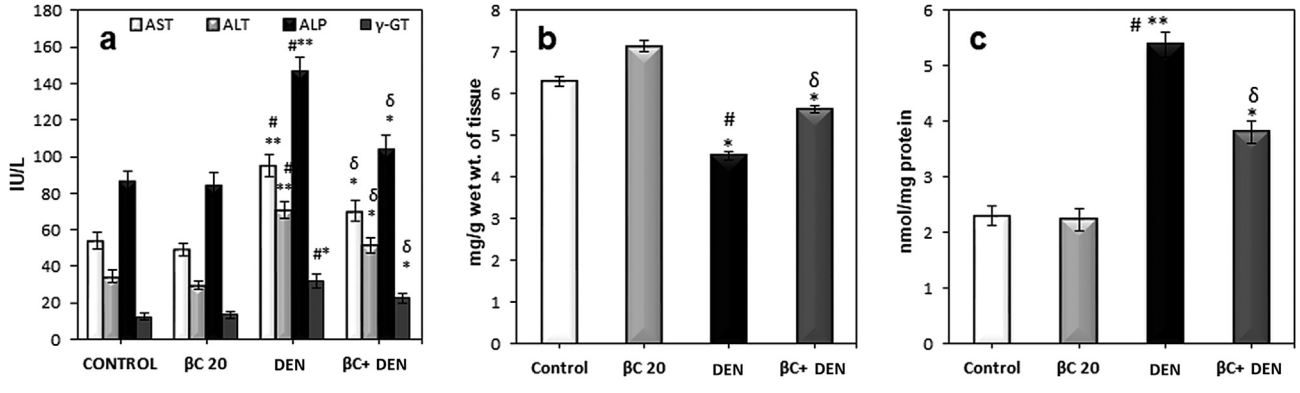

Figure 1. Effects of β-carotene on (a) Liver function test enzymes (IU/L) (b) Hepatic glycogen content (mg/g wet weight of tissue) and (c) Hepatic protein carbonyl content (nmoles/g of protein) (1) Control. (2) β-carotene 20 mg/kg body wt. treated rat. (3) NDEA treated and (4) Post treatment with β-carotene after 2 hrs of NDEA administration. The number of animals in each experimental group was five and experiments were performed in triplicates. $^{\#}$*P < 0.05, **P < 0.01 compared to normal control and $^{\delta}$*P < 0.05, **P < 0.01 compared to DEN treated group. Error bars represents standard error.

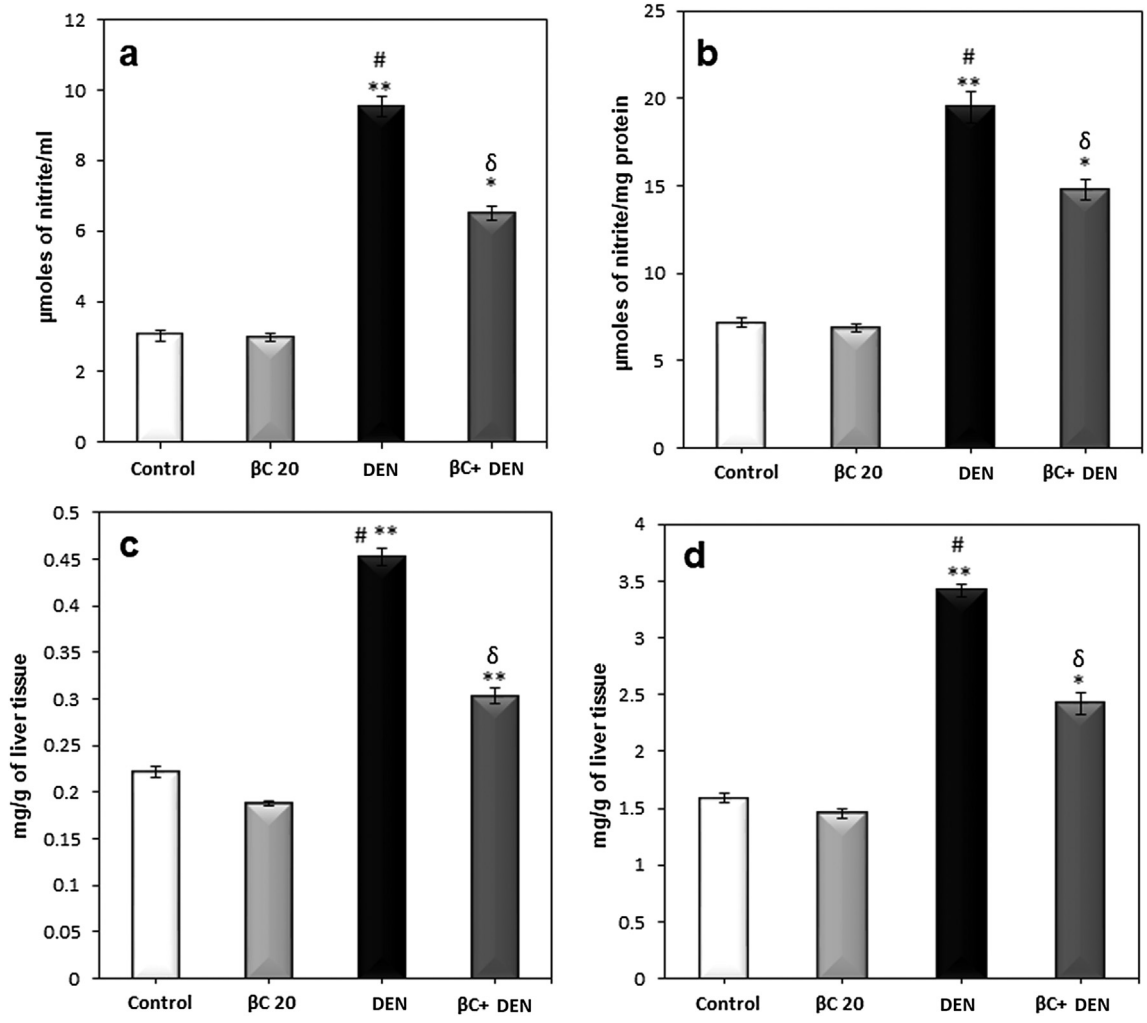

Figure 2. Effects of β-carotene on (a) Serum nitrite level (µmoles of nitrite/mL). (b) Hepatic nitrite level (µmoles of nitrite/mg protein). (c) Hepatic hydroxyproline content (mg/g of liver tissue) and (d) Hepatic collagen content (mg/g of liver tissue) (1) Control. (2) β-carotene 20 mg/kg body wt treated rat. (3) NDEA treated and (4) Post treatment with β-carotene after 2 hrs of NDEA administration. The number of animals in each experimental group was five and experiments were performed in triplicates. $^{\#}$*P < 0.05, **P < 0.01 compared to normal control and $^{\delta}$*P < 0.05, **P < 0.01 compared to DEN treated group. Error bars represents standard error.

respectively). β-Carotene supplementation significantly ($P < 0.05$) reversed these changes in hepatic protein carbonyls, sera and liver nitrite in the DEN-treated experimental rats within two weeks. To confirm fibrosis in the liver of DEN-treated rats and its amelioration by β-carotene, hepatic hydroxyproline and collagen contents were estimated. DEN-administered animals demonstrated significantly elevated levels of hydroxyproline as well as collagen (∼67 and ∼65%) in liver. However, these levels were significantly regressed by β-carotene administration which demonstrates its attenuating effect on the fibrotic changes in DEN-treated animals (Figures 2c and 2d).

Confocal microscopy of control liver specimen stained with Direct red displayed normal anatomy with perfect parenchyma and cell types (Figure 3). However, DEN-exposed fibrotic rats display increased collagen deposition in liver, particularly around portal areas and central vein. Administration of β-carotene reduced collagen fibers significantly which is further supported by hepatic hydroxyproline levels in these rats. Immuno-histochemical staining of iNOS was performed in the liver of different group of animals. In the rats belonging to control groups, weak signals of iNOS were detected in the liver sections (Figure 4). In DEN-administered group, iNOS staining intensified in the portal areas. However, β-carotene significantly reduced iNOS immune-positive cells in the liver.

Typical polypeptides pattern of soluble fractions from liver in the presence of sodium dodecyl sulphate are shown in Figure 5a. In the PAGE profiles iNOS and NF-κB resolved as single polypeptides of ∼131 and ∼60 kDa, respectively. The activities of iNOS and NF-κB were investigated in all animals by Western immunoblotting. DEN-induced

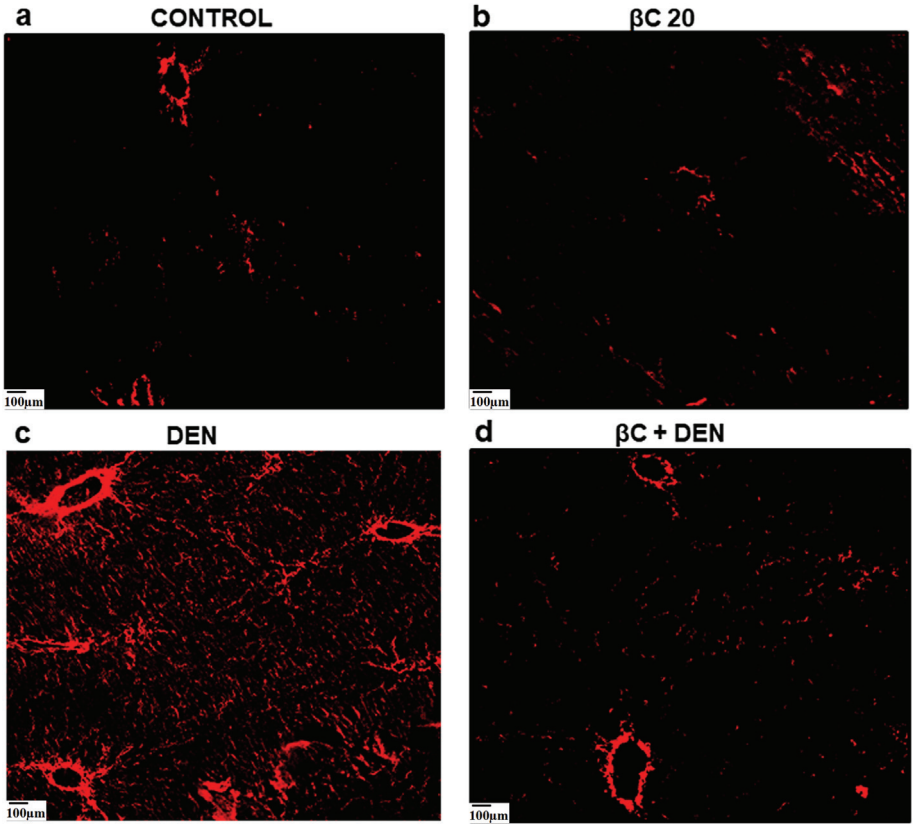

Figure 3. Picrosirius red staining showing collagen expression in hepatic tissue of rat under confocal microscope: (a) Control showing normal liver architecture with no collagen deposition (10×). (b) β-carotene 20 mg/kg body wt treated rat in which the results were comparable to normal control group (10×). (c) DEN treated showing collagen deposition around portal triad (10×). (d) Post treatment with β-carotene after 2 h of DEN administration shows residual collagen deposition around perivenular areas (10×).

fibrotic animals demonstrated significantly augmented activities of iNOS and NF-κB (Figures 5b and 5c). It is striking to note that both iNOS and NF-κB proteins were down-regulated in the liver of animals treated with β-carotene.

Discussion

β-Carotene possesses numerous powerful activities, such as free radicals scavenging, reducing inflammation and anti-cancerous [3, 37, 38]. β-Carotene can be taken from the bloodstream to be stored or readily metabolized by various tissues [39]. In mammals, liver is the central organ which accumulates enormous amount of β-carotene, followed by adipose tissue, kidney and skin. Liver can also acquire β-carotene to metabolize or package it in the form of very low density lipoproteins (VLDL). It has been previously demonstrated that β-carotene offers hepatoprotection against chemically-induced liver damage by declining α-SMA activity and inhibiting HSCs transformation [23]. However, the participation of inflammatory mediator, iNOS and NF-κB, in regulating DEN-induced liver injury was not so far evaluated. In this study, we report that β-carotene attenuates liver injury in rats by controlling iNOS expression *via* inhibiting NF-κB action.

Occurrence of hepatic injury in DEN treated rats is supported by deranged serum concentrations of the LFT biomarkers. An increase in the level of ALT is considered as a reliable signal of liver damage in rodents [40, 41]. Further, parenchymal injury causes spillage of transaminases (ALT/AST) from the cell cytosol into the blood circulation, and hence activities of these enzymes are high in the sera. It is opined that DEN metabolism causes release of free radicals which damages cellular membranes in liver. This injury consequently leads to seeping out of cytoplasmic contents into the systemic circulation. We noticed a decline in hepatic glycogen and an increase in protein carbonyl contents in the DEN administered rats. Glycogen is the main energy reserve of the body and an established indicator of liver functioning. Any decline in its level may be attributed either to decreased glycogenesis due to hepatocyte damage or increased glycogenolysis. The literature further suggests that free radicals mediate oxidation of proteins that results in the generation of free carbonyls [42], and for this reason protein carbonyl content is widely used to

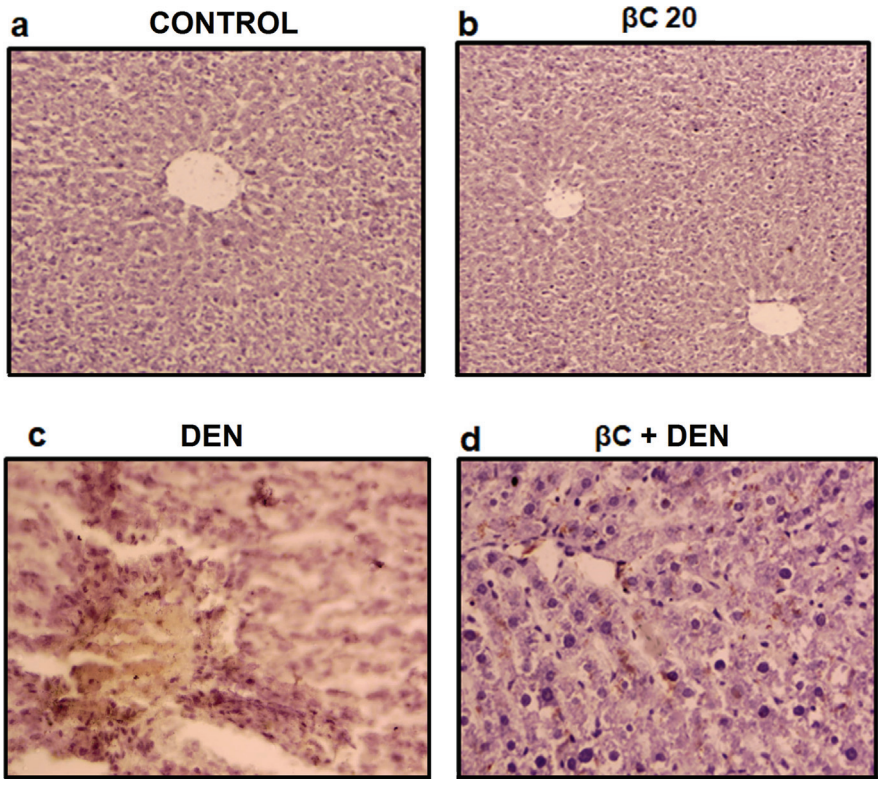

Figure 4. Immuno-histochemical staining of iNOS in the rat liver. (a) Normal control group (10 ×). (b) β-carotene 20 mg/kg body wt (10 ×). (c) DEN treated group showing iNOS positive cells (40 ×). (d) Post treatment of β-carotene after DEN administration (40 ×).

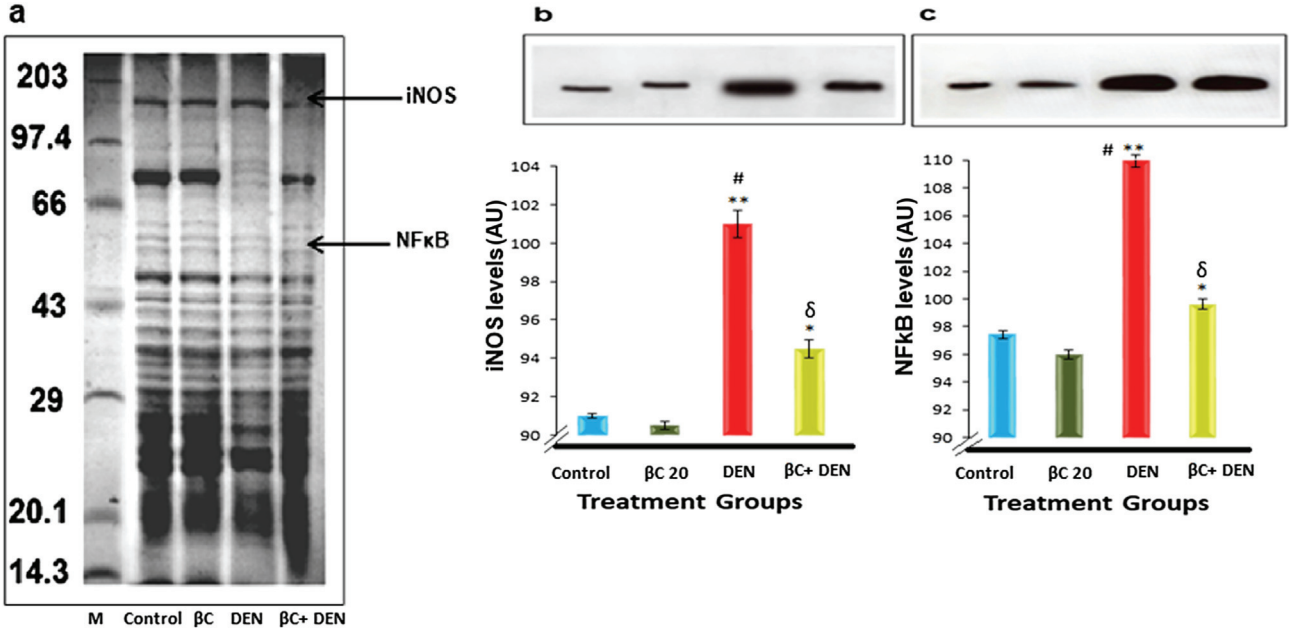

Figure 5. Effect of β-carotene on hepatic iNOS and NFκB in DEN treated rats. (a) Analysis by SDS PAGE. Immunoblot showing the expression of (b) iNOS and (c) NFκB in liver homogenates of: (1) Control. (2) β-carotene 20 mg/kg body wt treated rat. (3) NDEA treated and (4) Post treatment with β-carotene after 2 hrs of NDEA administration. All the values were taken as the mean ± standard error and experiments were performed in triplicates. $^{\#}$*P < 0.05, **P < 0.01 compared to normal control and $^{\delta}$*P < 0.05, **P < 0.01 compared to DEN treated group. Error bars represent standard error.

measure oxidative damage [43]. It is now apparent that DEN-induced free radical generation results in the increase in protein carbonyl contents. β-Carotene restitutes the LFT biomarkers, exerts hepatoprotective effect against free-radical inflicted damage and refurbishes hepatic glycogen levels within two weeks of its administration.

Published evidence shows that hydroxylation of prolines results in the production of hydroxyproline in the presence of prolyl hydroxylase in endoplasmic reticulum lumen. Hydroxyproline along with proline stabilizes collagen. The increase in this non-proteinogenic amino acid is also correlated with liver fibrosis [44]. Collagen, especially collagen type-I and -III, are principal constituents of ECM in the fibrotic tissue [45]. The evidence presented here clearly shows that liver detoxification and deterrence to collagen growth are concurrent phenomena. This is supported by the histopathology of DEN and β-carotene supplemented liver sections (picrosirius staining) of animals. It is due to lesser hydroxylation of collagen in β-carotene supplemented rats, depleted hydroxyproline levels were noted on day-14. It is apparent that β-carotene refurbished hydroxyproline by inhibiting collagenesis and elevating collagenolytic enzymes in the animal liver. Thus, administration of β-carotene significantly declines hydroxyproline and collagen levels, implying its anti-fibrogenic potential.

Nitrite is the primary oxidation product of nitric oxide (NO) subsequent to reaction with oxygen and, therefore, the increased nitrite concentration was used as an indicator of increased NO synthesis. NO is the main contributor of reactive nitrogen species (RNS). Specifically, peroxynitrite can damage macromolecules (i.e. proteins, lipids, DNA) and may result in protein nitration to affect structure and function of other target proteins [46]. A set of three different nitric oxide synthases (NOSs) catalyzes L-arginine to produce nitric oxide. iNOS being the main isoform of NOS in the liver, produces high amounts of NO [47]. Our results on biochemical assays, immunohistochemistry and western blotting reveal a significant increase in sera/liver nitrite levels and elevated activity of iNOS (Figures 2, 4 and 5b). It is thus concluded that the elevated NO in liver is due to upregulation of iNOS activity and these activities are directly correlated with the severity of liver damage, especially the necro-inflammation during DEN-induced hepatic fibrosis. Published evidence demonstrated that nuclear factor kappa-B (NF-κB) plays principal role in hepatic stellate cell activation and hepatic fibrosis [19, 48]. Reactive oxygen species generated by DEN can induce NF-κB activation and its subsequent nuclear translocation. NF-κB modulates liver injury by regulating production of inflammatory cytokine (such as TNF-α) and the induction of inflammation associated enzymes, including iNOS [49, 50]. β-Carotene treatment significantly decreases NF-κB activity and down-regulates nitrite and iNOS levels, thus ameliora- ting DEN-induced fibrosis in the liver. This may be due to blocking of IκBα degradation and subsequent translocation of NF-κB p65 subunit to the nucleus [38]. Thus, the mechanism of β-carotene suppressing NF-κB mediated inflammatory gene expressions operates *via* the inhibition of IκBα degradation.

Limitations and strengths

The only limitation of this study is that a small group of animals has been selected and rodents have limited scope of drug testing. Originality of the finding is that NF-κB can be regarded as a novel therapeutic target to prevent or even treat chemically induced hepatic fibrosis in humans.

Conclusion

This study unambiguously demonstrates that β-carotene ameliorated the advancement of liver fibrosis through several mechanisms. β-Carotene exercises its anti-fibrotic power by down-regulating NF-κB activity within fourteen days of its treatment in experimental rats. Thus, NF-κB inhibition might provide a new therapeutic strategy to mitigate liver fibrosis. However, more pre-clinical trials are needed to further determine the relationship between the therapeutic use of NF-κB signaling in hepatic fibrosis prevention.

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Conflict of interest
Authors of this manuscript declare no conflict of interest.

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