LIST OF PUBLICATIONS


RELATED PUBLICATIONS


2. Revathi Ramalingam, Manju Vaiyapuri, Umbelliferone with Vitamin C modulates lipid profile indices in diethylnitrosamine induced hepatocellular carcinoma. Journal of biological & scientific opinion (accepted)
The effects of umbelliferone on lipid peroxidation and antioxidant status in diethylnitrosamine-induced hepatocellular carcinoma

Revathi Ramalingam, Manju Vaiyapuri* Department of Biochemistry, Periyar University, Salem 11, Tamil Nadu, India Received 1 March 2013; accepted 10 May 2013

Abstract

Aim: Hepatocellular carcinoma (HCC) represents a major source of global mortality, still rising in worldwide. The present study aims at elucidating the antioxidant efficiency of umbelliferone (UMB) in N-Nitrosodimethylamine (DEN) induced rat liver carcinogenesis. Methods: Rats were randomly divided into six groups of six rats each as follows. Rats in group I received standard pellet diet and served as control, group II rats were induced with hepatocellular carcinoma by providing 0.01% DEN through drinking water 15 weeks. Group III rats received umbelliferone via intragastric intubation at a daily dose of 30 mg/kg body weight for 16 weeks every day. Groups IV&VI rats received 0.01% of DEN as in group II along with umbelliferone via intragastric intubation at a daily dose of 10, 20 and 30 mg/kg body weight for throughout the experimental period of 16 weeks. Results: DEN induction in experimental animals resulted in increased activities of liver marker enzymes and lipid peroxidation levels and decreased levels of antioxidant enzymes. Umbelliferone treatment restored the elevated activities of liver marker enzymes and antioxidant status to near-normal with decreased lipid peroxidation levels. Histological observations of liver tissue too correlated with the above biochemical findings. Conclusion: These results clearly suggest that umbelliferone treatment prevents liver damage, lipid peroxidation and protects the antioxidant defense system in DEN-induced liver carcinogenesis in rats. Copyright © 2013, Taiwan Society of Emergency Medicine. Published by Elsevier Taiwan LLC. All rights reserved.

Keywords: Antioxidants; Dimethyl nitrosamine; Hepatocellular carcinoma; Umbelliferone

1. Introduction

Primary hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the world.1 Accumulating evidence has suggested that several mechanisms contribute to the carcinogenesis of HCC.2,3 Recent efforts to control the incidence of HCC have focused on developing effective new chemoprevention strategies. HCC induced by diethylnitrosamine (DEN) in Wistar rats that shows similarities to human HCC is an ideal model for investigating the effect of intervention by chemopreventive agent.4 DEN, a hepatocarcinogen, is known to induce perturbations in the nuclear enzymes involved in DNA repair/replication.5 Investigations have provided evidence that DEN causes a wide range of tumors in all animal species, and these compounds are considered to be effective health hazards to man. Man is exposed to DEN through diet, in certain occupational settings, and through the use of tobacco products, cosmetics, pharmaceutical products, and agricultural chemicals.6 It has been reported that DEN, after its metabolic biotransformation, produces the promutagenic adducts, O2-ethyl deoxyguanosine and O2-ethyl deoxythymidine that can produce DNA chain damage, depurination or binding to DNA, and often generates a miscoding gene sequence, paving a way for the initiation of liver carcinogenesis.7 It has also

* Corresponding author. Department of Biochemistry, Periyar University, Periyar Palkalainagar, Salem 11, Tamil Nadu, India.
E-mail address: manjucb11@gmail.com (M. Vaiyapuri).

Please cite this article in press as: Ramalingam R, Vaiyapuri M, The effects of umbelliferone on lipid peroxidation and antioxidant status in diethylnitrosamine-induced hepatocellular carcinoma, Journal of Acute Medicine (2013), http://dx.doi.org/10.1016/j.jacme.2013.05.001
been reported to produce reactive oxygen species (ROS), a potentially dangerous by-product of cellular metabolism that may directly affect cellular development, growth, and survival.\(^7\) Oxidative stress caused by ROS has been reported in membrane lipid peroxidation, DNA damage, and mutation associated with the initiation of various stages of the tumor formation process.\(^9\)

Polyphenolic compounds have the most promising pharmacological properties and have received greater attention than any other class of natural products to counter the ill effects of oxygen radicals.\(^10\) Umbelliferone (UMB), otherwise known as 7-hydroxycoumarin, is a coumarin derivative of benzopyrone that is naturally present in edible fruits, such as Bengal quince (Aegle marmelos Correa)\(^11\) and bitter orange (Citrus aurantium).\(^12\) UMB is known to have a wide spectrum of pharmacological effects including antioxidant,\(^13\) antiabetic, antihyperglycemic,\(^14\) and anti-inflammatory properties.\(^14,15\) UMB acts as a fluorescent probe and is used in the synthesis of drugs, especially anticancer drugs, and in the treatment of asthma and allergic disorders. The ultraviolet activity of UMB is used as a sunscreen agent and an optical brightener for textiles. It has also been used as a gain medium for dye lasers. UMB can be used as a fluorescence indicator for metal ions such as copper and calcium. It acts as a pH indicator in the 6.5-8.9 range. It is acutely toxic to laboratory animals in chronic oral gavage administration at doses \(\geq 200\) mg/kg and exposure of UMB induces irritation of the eyes, respiratory system, and skin.

Therefore, the present study was designed to evaluate the anti-lipid peroxidation and antioxidant effects of UMB on DEN-induced hepatocellular carcinoma.

2. Materials and methods

2.1. Chemicals

UMB and DEN were purchased from Sigma-aldrich Chemical Company, Saint-Louis, MO, USA. All the other chemicals and reagents used were of analytical grade.

2.2. Tumor induction

HCC was induced in male Wistar rats by administering DEN at 200 mg/kg body weight in drinking water for 16 weeks.

2.3. Formulation and administration of umbelliferone

UMB was freshly prepared and dissolved in 10% dimethyl sulfoxide\(^16\) at a daily dose of 10 mg/kg, 20 mg/kg, and 30 mg/kg body weight.

2.4. Animal housing and diets

Male Wistar albino rats aged 8 weeks and weighing about 150-180 g were obtained from Sri Venkateshwara Enterprises, Bangalore, India. After 1 week of acclimatization, all rats were housed in groups of six per polypolyethylene plastic cage covered with metal grids and containing a hygienic bed of husk in a specific-pathogen free animal room under controlled conditions with a 12-hour light/12-hour dark cycle. They were provided with standard food pellets (diet composition: wheat broken moisture 9.0%, crude protein 11.5%, crude fat 1.9%, crude fiber 4%, ash 0.2%, and nitrogen-free extract 73.4%) supplied by Hindustan Lever Ltd, Mumbai, India, and tap water ad libitum. The study was conducted after obtaining clearance from the institutional animal ethical committee (Reg. No. P. Col/63/2011/IAEC/VMCP) of Vinayaka Mission College of Pharmacy, Salem, Tamil Nadu, India.

2.5. Study design and treatment schedule

Rats were randomly distributed into six groups of six rats each as follows. Rats in Group 1 received standard pellet diet and served as controls. Group 2 rats had hepatocellular carcinoma induced by providing 0.01% DEN through the drinking water for 15 weeks. Group 3 rats received UMB via intragastric intubation at a daily dose of 30 mg/kg body weight for 16 weeks. The rats in Groups 4 and received 0.01% DEN, as in Group 2, along with UMB via intragastric intubation at a daily dose of 10 mg/kg, 20 mg/kg, and 30 mg/kg body weight throughout the experimental period of 16 weeks. The experimental protocol is shown in Fig. 1. At the end of the experimental period, the rats were sacrificed by cervical dislocation and blood samples and liver tissue from the animals were taken for analysis.

2.6. Preparation of tissue homogenate and histopathological changes

After sacrifice, the liver tissue was macroscopically examined for the presence of tumors or other pathological lesions. Tissues with abnormal morphology were fixed in 10% buffered formalin and embedded in paraffin blocks. Histological sections stained with hematoxylin and eosin were used to confirm the presence and type of tumors by histopathological examination, which was performed by a pathologist unaware of the experimental codes. Liver tissue was removed immediately and washed with ice-cold saline and homogenized in the appropriate buffer in a tissue homogenizer.

2.7. Biochemical assessments

Lipid peroxidation was estimated by measuring the level of thiobarbituric acid reactive substances in the tissue via the method described by Ohkawa\(^16\) and plasma via Yagi’s method.\(^17\) The pink chromogen produced was measured at 532 nm. The values are expressed as mmol/100 g tissue or mmol/
mg Hb. The level of conjugated dienes was assessed using the method described by Rao and Recknagel. This method is based on the arrangement of the double bonds in polyunsaturated fatty acids to form conjugated dienes with an absorbance maximum at 233 nm. The lipid hydroperoxide contents were measured using the method described by Jiang et al. Hydroperoxides are detected by their ability to oxidize ferrous iron leading to the formation of a chromophore with an absorbance maximum at 560 nm. The level of lipid peroxides was expressed as nmol malonaldehyde/mg protein/nmol/mL Hb. Reduced glutathione (GSH) content was determined via the method given by Ellman. GSH determination is based on the development of a yellow color when 5,5′-dithio(2-nitrobenzoic acid) (DTNB) is added to compounds containing sulphydryl groups. Glutathione peroxidase (GPx, EC.1.11.1.9) activity was assayed via using the method described by Rotruck et al with a modification. A known amount of enzyme preparation was incubated with hydrogen peroxide in the presence of GSH for a specified time period. The amount of hydrogen peroxide utilized was determined using the method given by Ellman. The values are expressed as mmol GSH utilized/min/mg protein. The glutathione-S-transferase (GST, EC. 2.5.1.18) activity was estimated via the method given in Habig et al by following the increase in absorbance at 340 nm using 1-chloro-2, 4-dinitrobenzene as the substrate. Glutathione reductase activity was assayed using the method described by Carlberg and Mannervik by measuring the GSH formed by nicotinamide adenine dinucleotide phosphate (NADPH).

Superoxide dismutase (SOD, EC.1.15.1.1) was assayed using the Kakkar et al’s method based on the 50% inhibition of the formation of NADH phenazine methosulfate nitroblue tetracodium formazan at 520 nm. One unit of the enzyme is taken as the amount of enzyme required for 50% inhibition of nitro blue tetracodium (NBT) reduction/min/mg protein. The activity of catalase (CAT, EC.1.1.1.16) was determined via the method given by Sinha. Dichromate in acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide with the formation of perchromic acid as an unstable CAT intermediate. The chromic acetate formed was measured at 590 nm. CAT was allowed to split hydrogen peroxide for different periods of time. The reaction was stopped at different time intervals by the addition of a dichromate-acetic acid mixture, and heating the reaction mixture and measuring chromic acetate colorimetrically determined the remaining hydrogen peroxide.

Vitamin C (ascorbic acid) content was estimated using the method given by Roe and Kuether in which dehydroascorbic acid is coupled with 2,4-dinitrophenylhydrazine and then treated with sulfuric acid, forming an orange-red colored compound, the content of which was measured at 520 nm. Vitamin E (α-tocopherol) content was estimated using the methods of Barker and Frank. The method involves the...
α-tocopherol-mediated reduction of ferric ions to ferrous ions, and the formation of a red colored complex with 2,2′-dipyridyl. The absorbance of the chromophore was measured at 520 nm. The activities of the marker enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were assayed using King’s methods.

2.8. Statistical analysis

Values are given as the mean ± standard deviation. The significant differences between the means of the six groups were statistically analyzed by one-way analysis of variance (ANOVA) and the Kruskal Wallis test with the Mann Whitney test. The significance levels were set at p < 0.001 for all of the tests. Statistical analyses were performed using SPSS 16.0 software package (SPSS, IBM product, Chicago, IL, USA).

3. Results

Microscopic observations of UMB in DEN-treated rat liver are given in Fig. 2. Fig. 2 shows the histopathological examination of liver section. Control (Group 1) rats revealed normal liver parenchyma cells with granulated cytoplasm, small uniform nuclei, and central vein surrounded by cords of hepatocytes. Group 2 DEN-treated rats showed loss of architecture and lobules of neoplastic hepatocytes with a fecal area of fatty change. Group 3 rats exhibited normal architecture, indicating the non-toxic nature of UMB. Groups 4 and 5 rats co-treated with UMB and DEN showed moderate cancerous change, fatty change, and hydropic degeneration. Group 6 rats showed fewer neoplastically-transformed cells and the hepatocytes maintained near-normal architecture.

3.1. Effect of UMB on lipid peroxidation levels in both serum and liver

Fig. 3 shows the levels of lipid peroxidation both in plasma and liver of control (Group 1) and experimental groups. Significantly increased levels of lipid peroxidation were observed in DEN-induced liver cancer-bearing animals (Group 2). Administration of UMB to DEN-induced rats (Groups 4, 5, and 6) significantly decreases the lipid peroxidation level, which was brought to near-normal. There were no significant differences observed in UMB- treated (Group 3) and control rats (Group 1).

3.2. Effect of UMB on the antioxidant defense system

Figs. 4 and 5 show the levels of plasma and liver tissue enzymatic antioxidants [SOD, CAT, GPx, glutathione reductase (GR), and GST] in control and experimental rats. The control rats (Group 1) had normal levels of these enzymes; whereas HCC-induced rats (Group 2) showed significantly reduced levels when compared to other groups. UMB given alone (Group 3) highlights the increased levels of these enzymes when compared to control rats. The administration of UMB to DEN-induced rats (Groups 4, 5, and 6) restored the changes to near-normal levels due to the antioxidant efficacy of UMB.
3.3. Effect of UMB on hepatic marker enzymes (AST, ALT, ALP, and LDH)

Table 1 shows the levels of the tissue hepatic marker enzymes (AST, ALT, ALP, and LDH) of control and experimental rats. DEN-induced rats (Group 2) exhibited a significant elevation in the activity of these marker enzymes when compared to control rats (Group 1); whereas UMB-treated rats (co-treated with UMB and DEN, Groups 4, 5, and 6) showed a significant decrease in the levels of these marker enzymes when compared with DEN-induced rats.

Table 2 gives the levels of the serum marker enzymes (AST, ALT, ALP, and LDH) of control and experimental rats. DEN-administered rats (Group 2) showed a significant increase in the activity of these marker enzymes when compared to control rats.
Effect of umbelliferone on circulatory antioxidant enzymes of control and experimental rats.

Data are presented as the means ± SD of each group. P < 0.001 among the six groups (Kruskal Wallis Test). *enzyme required for 50% inhibition of NBT reduction/min/mg of Hb. †μmol H$_2$O$_2$ utilized/min/mg of Hb. ‡μmoles of GSH utilized/min/mg of Hb. §μmoles of GSH utilized/min/mg of Hb. ¶μmoles of DTNB-GSH conjugate formed/min/mg of Hb).

SOD: Group I vs III P = 0.008. I vs IV P = 0.006. I vs all other groups P = 0.004. II vs IV P = 0.006. CAT: I vs IV P = 0.006. I vs all other groups P = 0.004. II vs IV P = 0.006. II vs all other groups P = 0.004. Gpx: I vs IV P = 0.006. I vs all other groups P = 0.004. II vs IV P = 0.006. II vs all other groups P = 0.004. Gf: I vs III P = 0.873. I vs IV P = 0.006. I vs all other groups P = 0.004. II vs III P = 0.173. I vs IV P = 0.006. I vs all other groups P = 0.004. II vs IV P = 0.006. II vs VI P = 1.00. II vs all other groups P = 0.064 (Mann Whitney Test).

Fig. 5. Effect of umbelliferone on circulatory antioxidant enzymes of control and experimental rats.
control rats (Group 1), whereas DEN-induced rats treated with UMB (Groups 4, 5, and 6) showed a drastic decline in the levels of these marker enzymes when compared with DEN-induced rats (Group 2).

3.4. Effect of UMB on non enzymatic antioxidant status in control and experimental rats

Table 3 shows the levels of hepatic tissue non-enzymatic antioxidants (vitamin C, vitamin E, and GSH) in control and experimental rats. The enzyme levels of control rats (Group 1) were normal whereas the levels in HCC-induced rats (Group 2) were significantly reduced when compared to other groups. UMB alone (Group 2) showed increased levels of these enzymes when compared to control rats. In the rats co-treated with UMB and DEN (Groups 4, 5, and 6), antioxidant levels were restored to near-normal by the antioxidant efficacy of UMB.

4. Discussion

In recent years, there has been a growing interest in dietary substances obtained from natural products having chemoprotective properties against chemical carcinogens. HCC is a common cancer and is the third leading cause of death worldwide. DEN is known to induce the reproducible and complete carcinogenic biochemical changes involved in the progression of HCC. ROS are potentially dangerous byproducts of cellular metabolism that have directly affected cellular growth, development, and survival. Lipid peroxidation is one of the major mechanisms of cellular injury caused by free radicals and acts as an important causative factor in carcinogenesis. DEN intoxication has been reported to generate lipid peroxidation byproducts that may interact with various biomolecules that lead to oxidative stress. This may be due to the uncontrolled generation of free radicals that overwhelms the antioxidant defense system. DEN-induced rats showed increased lipid peroxidation levels (thiobarbituric acid reactive

Table 1
The effects of umbelliferone on the hepatic tissue marker enzymes of control and experimental rats.

<table>
<thead>
<tr>
<th></th>
<th>Aspartate transaminase (IU/L)</th>
<th>Alanine aminotransferase (IU/L)</th>
<th>Alkaline phosphatase (IU/L)</th>
<th>Lactate dehydrogenase (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>92.82 T 0.15</td>
<td>84.52 T 0.27</td>
<td>87.69 T 0.21</td>
<td>87.71 T 0.20</td>
</tr>
<tr>
<td>DEN</td>
<td>132.46 T 0.24</td>
<td>122.65 T 0.23</td>
<td>138.80 T 0.07</td>
<td>138.65 T 0.22</td>
</tr>
<tr>
<td>UMB 30 mg/kg</td>
<td>91.57 T 0.18</td>
<td>86.58 T 0.26</td>
<td>89.63 T 0.25</td>
<td>86.68 T 0.20</td>
</tr>
<tr>
<td>DEN þ UMB 10 mg/kg</td>
<td>124.65 T 0.22</td>
<td>120.62 T 0.26</td>
<td>129.66 T 0.25</td>
<td>129.77 T 0.19</td>
</tr>
<tr>
<td>DEN þ UMB 20 mg/kg</td>
<td>118.60 T 0.18</td>
<td>110.67 T 0.23</td>
<td>110.68 T 0.21</td>
<td>110.68 T 0.20</td>
</tr>
<tr>
<td>DEN þ UMB 30 mg/kg</td>
<td>101.93 T 0.04</td>
<td>99.96 T 0.01</td>
<td>98.78 T 0.18</td>
<td>98.70 T 0.18</td>
</tr>
</tbody>
</table>

Data are presented as the means T SD of each group. P < 0.001 among the six groups (Kruskal Wallis Test). P ¼ 0.004 (Mann Whitney Test).

Table 2
The effects of umbelliferone on the serum hepatic marker enzymes of control and experimental rats.

<table>
<thead>
<tr>
<th></th>
<th>Aspartate transaminase (IU/L)</th>
<th>Alanine aminotransferase (IU/L)</th>
<th>Alkaline phosphatase (IU/L)</th>
<th>Lactate dehydrogenase (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>121.70 T 0.21</td>
<td>41.68 T 0.19</td>
<td>36.57 T 0.30</td>
<td>121.63 T 0.21</td>
</tr>
<tr>
<td>DEN</td>
<td>275.58 T 0.16</td>
<td>103.72 T 0.24</td>
<td>95.76 T 0.13</td>
<td>275.58 T 0.17</td>
</tr>
<tr>
<td>UMB 30 mg/kg</td>
<td>124.74 T 0.19</td>
<td>42.64 T 0.26</td>
<td>38.61 T 0.28</td>
<td>120.41 T 0.36</td>
</tr>
<tr>
<td>DEN þ UMB 10 mg/kg</td>
<td>127.76 T 0.23</td>
<td>41.80 T 0.15</td>
<td>35.94 T 0.03</td>
<td>170.52 T 0.24</td>
</tr>
<tr>
<td>DEN þ UMB 20 mg/kg</td>
<td>128.71 T 0.23</td>
<td>43.69 T 0.22</td>
<td>35.93 T 0.01</td>
<td>156.44 T 0.43</td>
</tr>
<tr>
<td>DEN þ UMB 30 mg/kg</td>
<td>131.67 T 0.20</td>
<td>45.11 T 0.60</td>
<td>37.65 T 0.28</td>
<td>131.55 T 0.24</td>
</tr>
</tbody>
</table>

Data are presented as the means T SD of each group. P < 0.001 among the six groups (Kruskal Wallis Test). P ¼ 0.004 (Mann Whitney Test).

Table 3
The effects of umbelliferone on liver tissue and the plasma non-enzymatic antioxidant status of control and experimental rats.

<table>
<thead>
<tr>
<th></th>
<th>Tissue GSH (nm/g)</th>
<th>Plasma GSH (nm/g)</th>
<th>Vitamin C (mg/mg protein)</th>
<th>Vitamin E (mg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.27 T 0.02</td>
<td>4.83 T 0.01</td>
<td>0.753 T 0.02</td>
<td>0.461 T 0.02</td>
</tr>
<tr>
<td>DEN</td>
<td>11.15 T 0.03</td>
<td>2.65 T 0.007</td>
<td>0.346 T 0.02</td>
<td>0.163 T 0.02</td>
</tr>
<tr>
<td>UMB 30 mg/kg</td>
<td>16.25 T 0.02</td>
<td>4.97 T 0.011</td>
<td>0.846 T 0.02</td>
<td>0.460 T 0.01</td>
</tr>
<tr>
<td>DEN þ UMB 10 mg/kg</td>
<td>12.84 T 0.03</td>
<td>2.01 T 0.012</td>
<td>0.406 T 0.02</td>
<td>0.240 T 0.01</td>
</tr>
<tr>
<td>DEN þ UMB 20 mg/kg</td>
<td>15.73 T 0.03</td>
<td>2.08 T 0.005</td>
<td>0.506 T 0.01</td>
<td>0.280 T 0.08</td>
</tr>
<tr>
<td>DEN þ UMB 30 mg/kg</td>
<td>17.05 T 0.04</td>
<td>2.48 T 0.008</td>
<td>0.603 T 0.03</td>
<td>0.324 T 0.02</td>
</tr>
</tbody>
</table>

Data are presented as the means T SD of each group. P < 0.001 among the six groups (Kruskal Wallis test). Tissue GSH: Group I vs II, III P ¼ 0.004. I vs IV P ¼ 0.006. I vs V, VI P ¼ 0.003. II vs I, III P ¼ 0.004. II vs IV P ¼ 0.005. II vs V, VI P ¼ 0.003. Plasma GSH: Group I vs II, III, V P ¼ 0.004. I vs IV P ¼ 0.006. I vs V, VI P ¼ 0.054. II vs I, III, V, VI P ¼ 0.004. II vs IV P ¼ 0.006. II vs IV P ¼ 0.006. Vitamin C: P ¼ 0.004 with in the six groups. Vitamin E: I vs III P ¼ 0.871. I vs all other groups P ¼ 0.004. II vs all other groups P ¼ 0.004 (Mann Whitney Test).
Fig. 6. Effect of umbellferone on liver tissue antioxidant enzymes of control and experimental rats.

Please cite this article in press as: Ramalingam R, Vaiyapuri M, The effects of umbellferone on lipid peroxidation and antioxidant status in diethylnitrosamine-induced hepatocellular carcinoma, Journal of Acute Medicine (2013), http://dx.doi.org/10.1016/j.jacme.2013.05.001
substances, malondialdehyde and conjugated dienes) in both plasma and liver tissue. Umbelliferone administration to DEN-treated rats at three different doses 10 mg/kg, 20 mg/kg, and 30 mg/kg body weight every day led to significantly decreased levels of lipid peroxidation both in the plasma and liver when compared with animals induced with DEN alone. This shows the antilipid peroxidative role of UMB and is probably mediated by UMB’s ability to inhibit free radical generation. These results also correlate with the previous findings from our laboratory. The strong inhibitory effect of UMB at a dose of 30 mg/kg body weight/day was noticed.

Naturally-occurring antioxidants induce a variety of biological activities, including the induction of drug-metabolizing enzymes, inhibiting carcinogen-induced mutagenesis, and scavenging of free radicals. The development of life-threatening diseases like cancer is linked to the availability of these antioxidants. Chemical induction of hepatic carcinoma is associated with changes in oxygen radical metabolism. This change was demonstrated by a measurement of the antioxidant enzymes. Tumor cells have abnormal antioxidant enzyme activities. In the present study, the cancer-bearing rats showed decreased activities of enzyme antioxidants (SOD, CAT, GPx, GST, and GR) and non-enzyme antioxidants (GSH, vitamin E, and vitamin C) in both plasma and liver tissue. Our present data are consistent with previous findings.

Daily UMB supplementation given to DEN-treated rats at three different doses (10 mg/kg, 20 mg/kg, and 30 mg/kg body weight) significantly increased all of the above antioxidants, which may be due to the ability of UMB to interact with radicals, thereby subsequently scavenging them. This is because it donates electrons to unstable oxidized molecules, in turn reducing the free radicals. It also converts inactive antioxidant enzymes into active ones, thereby increasing the concentrations of antioxidant enzymes in the tissues. UMB has one hydroxyl and one acetoxy group in the benzoid ring (see Fig. 6), which may be responsible for its antioxidant and radical scavenging properties. A previous report has shown that UMB has an alkylperoxy radical scavenging property and it has greater intensity and activity against ROS and activates the antioxidant enzymes that are inactivated by ROS during peroxidation. It is therefore suggested that UMB treatment could protect normal cell or tissues against the cytotoxic effects of carcinogens. The strong inhibitory effect of UMB at a dose of 30 mg/kg body weight was noticed.

Liver damage caused by DEN generally reflects the instability of liver cell metabolism, which leads to distinctive changes in hepatospecific enzymes such as aminotransferases, phosphates, and LDH, and these enzymes leak from the damaged tissues into the body fluids due to their tissue specificity and catalytic activity. These enzymes are representative of liver function, so they are considered to be sensitive and dramatic indicators of hepatic injury and loss of functional integrity of the membrane. Transaminases (AST and ALT) are reliable, first marker enzymes of the liver, and are used in diagnostic enzymology. ALP is another important key marker enzyme located in the bile canaliculi membrane, so any interference with the bile flow (whether extra- or intrahepatic) leads to an alteration in these enzymes. LDH is a fairly sensitive marker of solid neoplasms. DEN-induced hepatic damage is usually accompanied by a rise of AST, ALT, ALP, and LDH due to the overproduction of these enzymes in tumor cells, which may cause increased permeability of the cell membrane, resulting in DEN intoxication. UMB administration to DEN-treated rats at three different doses (10 mg/kg, 20 mg/kg, and 30 mg/kg body weight) reverses the changes in liver-specific enzyme levels both in the serum and tissue. UMB treatment may significantly attenuate the increased activities of these enzymes, which may be due to the ability of UMB to protect the cells from membrane damage and maintain membrane integrity, thereby decreasing enzyme leakages.

In conclusion, the present study clearly indicates that the administration of UMB at a dose of 30 mg/kg body weight appreciably attenuates the reversible alterations in lipid peroxidation and overall enzymatic antioxidant status and that UMB reduces liver-specific enzyme leakage from the tissue of DEN-induced rat models. However, further studies are required to elucidate the molecular mechanism of UMB.

Acknowledgments

Financial support from a Senior Research Fellowship from the Indian Council of Medical Research (ICMR), Government of India, and New Delhi is gratefully acknowledged.

References