CURCUMIN AND NARINGIN PREVENT 7,12-DIMETHYLBENZ(A)ANTHRACENE-INDUCED HEPATIC INJURY BY SUPPRESSING INFLAMMATION AND OXIDATIVE STRESS

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ABSTRACT
The present study was carried out to evaluate the preventive effects of curcumin and/or naringin on 7,12-dimethylbenz(a)anthracene (DMBA)-administered rats. DMBA was administered by gastric intubation at a single dose of 25 mg/kg b.w. Curcumin and/or naringin were supplemented to DMBA-administered rats by gastric intubation at dose level of 25mg/kg b.w./day for 2 and 6 weeks beginning from the 1st day of DMBA administration. DMBA-administered rats exhibited a profound increase in serum AST, ALT, ALP and GGT activities as well as decrease in serum albumin level. Liver lipid peroxidation was elevated while liver glutathione content and glutathione-S-transferase activity were markedly decreased in DMBA-administered rats. Curcumin and/or naringin treatments successfully prevented DMBA-induced elevation in serum AST, ALT, ALP and GGT activities as well as decrease in serum albumin level. They also enhanced the deterioration in liver glutathione content and glutathione-S-transferase activity and inhibited the DMBA-induced liver lipid peroxidation, serum TNF-α and serum α-fetoprotein. The administration of curcumin and naringin to DMBA-treated rats successfully improved the liver histological perturbations which include diffuse fatty changes, degenerative changes, necrosis, inflammatory cells' infiltration and dilated and congested central and portal veins at 2nd and 6th weeks of the experiment. Hence, these results clearly suggest that curcumin and/or naringin treatments may counteract DMBA-induced effects on liver injury via enhancement of liver antioxidant defense system and suppression of oxidative stress and inflammation.

KEYWORDS: Curcumin; Naringin; DMBA; Liver Injury; Inflammation; Oxidative Stress.

1. INTRODUCTION
Liver is the central organ of metabolism and acts as an organ of storage and also, hepatic cells metabolize many potentially toxic substances (Muqbil and Banu, 2006). The great
susceptibility of liver to damage by chemical agents is presumably a consequence of its primary role in the metabolism of xenobiotics (Al-Athar, 2004). Hepatotoxicity is characterized by liver enzyme elevations and/or the presence of signs or symptoms signifying such injury, including nausea, vomiting, jaundice or lower extremity edema (Sass and Shakil, 2006). Drug-induced hepatotoxicity is a common cause of liver injury since it accounts for approximately one-half of the causes of acute liver failure and mimics all forms of acute and chronic liver disease (Kaplowitz, 2001; Ostapowicz et al., 2002; McNally, 2006). Polycyclic aromatic hydrocarbons (PAHs) are contaminants that may enter aquatic systems through spillage and seepage of fossil fuels, discharge of domestic and industrial wastes, atmospheric input and continental run off (Hartl, 2002). Their presence in the environment is of concern since they induce acute toxicity in organisms and have been linked to liver neoplasms and other abnormalities in fish species (Jha, 2004). 7,12-Dimethylbenzanthracene (DMBA), a polycyclic aromatic hydrocarbon (PAH) in which the methyl substitution greatly enhances carcinogenicity and toxicity (Smithgall et al., 1988), is produced during the incomplete combustion of carbon-containing compounds, and is predominantly found in tobacco smoke and motor vehicle exhaust emissions (Girolami et al., 2008). Exposure to PAHs, including DMBA, can lead to toxicological changes in the liver including oxidative stress and production of carcinogenic metabolites (DiGiovani and Juchau 1980). Several studies have focused on the effects of DMBA on biochemical and antioxidant parameters in the liver (Muqbil and Banu 2006; Choi, 2008; Girolami et al. 2008).

Curcumin (1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is the yellow pigment found in the rhizome of the plant Curcuma longa, also known as turmeric. It has shown to be non-toxic and non-mutagenic (Aggarwal et al., 2003) and exhibits a wide spectrum of biological activities, which include anti-inflammatory, anti-oxidant, anti-carcinogenic, antimutagenic, anticoagulant, anti-fibrotic, anti-diabetic, anti-bacterial, anti-fungal, antiviral, anti-venom, anti-ulcer, hypotensive, hypercholesterolemic, and cardioprotective activities (Ahmed, 2005; Ahmed and Adbel-Rehem, 2005; Naik et al., 2011). Curcumin has been found to protect against drug-induced toxicity as well such as adriamycin nephrotoxicity (Venkatesan, 2000) and alcohol-induced liver injury (Nanji et al., 2003). Naringin (4,5,7-trihydroxyflavanone-7-rhamnoglucoside) is a major and active flavanone glycoside of grape fruit and many citrus herbs (Jagetia and Reddy, 2002). Naringin is reported to possess antilucer, superoxide scavenging, and antioxidant activities (Kroyer, 1986 and Chen et al., 1990). Naringin exhibits various pharmacological and therapeutic
properties including anti-inflammatory (Nie et al., 2012; Tsai et al., 2012), antiulcer (Jagetia et al., 2003), antitussive (Gao et al., 2011; Luo et al., 2012), anti-carcinogenic (Qin et al., 2011 and Camargo et al., 2012), anti-fibrosis (Du et al., 2009), free radical scavenging and antioxidant (Jagetia and Reddy, 2005; Rajadurai and Prince, 2009; Gopinath and Sudhandiran, 2012) and anti-diabetic effects (Ahmed et al., 2012). Therefore, the present study was designed to assess the possible preventive effects of curcumin and/or naringin against DMBA-induced hepatotoxicity.

2. Materials and methods:

2.1. Drugs and Chemicals:
DMBA, curcumin and naringin were purchased from Sigma Chemical Company, St. Louis, MO, USA. They were stored at 2-4°C and protected from sunlight. All other chemicals used in this experiment were of analytical grade. The dose selection for each compound was based on previously published studies.

2.2. Experimental Animals:
Male albino rats weighing between 100-120g were used as experimental animals in the present investigation. They were obtained from the Animal House of Abou Rawash, El-Giza, Egypt. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in plastic cages with good aerated covers at normal atmospheric temperature (25±5°C) as well as under good ventilation and received water and standard balanced diet ad libitum. All animal procedures are in accordance with the recommendations for the proper care and use of laboratory animals of Canadian Council on Animal Care (Canadian Council on Animal Care [CCAC], 1993). All attempts were done to reduce the number of used animals and their suffering.

2.3. Doses and Treatment:
The dose of DMBA used in this study was 25 mg/kg b.w. (Shimki et al., 1969). It was dissolved in 5 ml of filter sterilized heavy olive oil, mixed well and administered as single dose by gastric intubation (Han et al., 2002). Curcumin and naringin were administered either alone or in combination to male albino rats at dose 25 mg / kg. b.w. / day (Zhang et al., 2000; El-Makawy and Sharaf, 2006) for 8 weeks by gastric intubation. They were dissolved in 1%
CMC (carboxymethylcellulose) as a vehicle at concentration 25mg curcumin and/or naringin/5ml CMC (1%).

2.4. Experimental design:
The number of rats used in the present study is 60. They were allocated into 5 groups designed as follow:

**Group 1:** This group was regarded as normal control group and was given the equivalent volume of olive oil as a single dose and 1% CMC daily by gastric intubation for 6 weeks.

**Group 2:** It was given single dose of DMBA 25 mg/kg b. w. dissolved in olive oil at beginning of the experiment by gastric intubation and then given the equivalent volume of 1% CMC daily for 6 weeks.

**Group 3:** The rats of this group were administered single dose of DMBA (25 mg/kg b. w.) at beginning of the experiment and were also treated with curcumin (25 mg /kg b. w. /day), dissolved in CMC, by gastric intubation for 6 weeks.

**Group 4:** The rats of this group were administered single dose of DMBA (25 mg/kg b. w.) at beginning of the experiment and also treated with naringin (25 mg /kg b. w./day), dissolved in 1% CMC, by gastric intubation for 6 weeks.

**Group 5:** The rats included in this group were administered single dose of DMBA (25 mg/kg b. w.) at beginning of the experiment and also treated with curcumin (25 mg /kg b. w./day) and naringin (25 mg /kg b.w./day), dissolved in 1% CMC, by gastric intubation for 6 weeks. At the end of 2 and 6 weeks, six animals of normal, DMBA-administered control rats and DMBA-administered rats treated with curcumin and/or naringin were sacrificed under mild diethyl ether anesthesia. Blood from each rat was collected from jugular vein in a centrifuge tube and left to clot at room temperature for 45 minutes. Sera were separated by centrifugation at 3000 r.p.m. at 30°C for 15 minutes and kept frozen at -30°C pending biochemical analyses. Liver from each animal was rapidly excised after dissection. One part was fixed in neutral buffered formalin for 24 hrs, trimmed and then transferred into 70%
alcohol for histopathological examination. 0.5g was homogenized in 5ml 0.9% sterilized NaCl (10% w/v) using teflon homogenizer (Glas-Col, Terre Haute, USA).

2.5. Biochemical analyses:

Alanine Aminotransferase (ALT) and Aspartate aminotransferase (AST) activities in serum was measured by kinetic method using kits obtained from BioSystem Company (Spain) according to Bertis et al. (2005). Alkaline phosphatase (ALP) activity in serum was measured by kinetic method using reagent kits obtained from BioSystem Company (Spain) according to Friedman and Young (1992). Serum albumin concentration was measured by colorimetric method using reagent kits purchased from Diamond Diagnostics Company (Egypt) according to Doumas et al., (1971). Gamma glutamyl transferase (GGT) activity in serum was measured by colorimetric method using kits developed by Spectrum Diagnostics according to Szasz et al. (1974). Serum Tumor necrosis factor-α (TNF-α) and α-fetoprotein (AFP) were measured by colorimetric method using kits developed by Quantikine 614 McKinley Place NE, USA according to manufacturer’s instructions. Liver glutathione (GSH) was determined according to the method of Beulter et al. (1963). Liver lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) according to the method of Preuss et al. (1998). Glutathione-S-transferase activity in liver was determined according to Mannervik and Gutenberg, (1981).

2.6. Histopathological studies:

Liver from each rat was immediately excised, washed using chilled saline solution and blotted. A small piece of each was immediately fixed in 10% formalin. The fixed organs were transferred to National Cancer Institute (NCI), Cairo University, Egypt. These formalin-fixed tissues were embedded in paraffin, sectioned (5μm), stained with hematoxylin and eosin (H&E), and examined under a light microscope for histopathological assessment.

2.7. Statistical analysis:
The data were analyzed using the one-way analysis of variance (ANOVA) (PC-STAT, University of Georgia, 1985) followed by LSD analysis to compare various groups with each other. Results were expressed as mean ± standard error (SE). Values of P>0.05 were considered non-significantly different, while those of P<0.05, P<0.01 and P<0.001 were considered significant highly and very highly significant different, respectively.
3. Results:

3.1. Effects on biochemical and oxidative stress parameters:

Changes in different serum enzymes related to liver function are represented in table 1. Serum AST, ALT and ALP activities were significantly (p<0.05) increased as a result of administration of DMBA alone at the 2nd and 6th weeks. These enzyme activities were decreased in the rats administered DMBA and treated with curcumin and/or naringin. The treatment with mixture of curcumin and naringin seemed to be more effective in improving the elevated AST, ALT and ALP activities of DMBA-administered rats. Serum GGT activity, on the other hand, was highly significantly (P<0.01; LSD) elevated in DMBA-administered rats at the end of the 2nd week and it was non-significantly (P>0.05; LSD) affected at the 2nd week. However, while the treatment with curcumin and the mixture of curcumin and naringin successfully (P<0.01; LSD) improved the elevated GGT activity at the 2nd week of DMBA-administration, naringin alone failed (P>0.05; LSD). Moreover, the curcumin administration seemed to be more potent than its mixture with naringin.

On the other hand, serum albumin level (table 2) was remarkably decreased in DMBA-administered rats at the 2nd and 6th weeks. The treatment of DMBA-administered rats with curcumin and/or naringin potentially ameliorated the lowered serum albumin level. Also, changes in serum Tumor Necrosis Factor-α (TNF-α) and α-fetoprotein (AFP) are represented in table 2. Serum TNF-α and AFP levels were highly significantly (p<0.01) elevated in DMBA-administered rats and highly significantly (p<0.01) decreased as a result of the treatments with curcumin and/or naringin; the combination of both tested agents seemed to be more effective in decreasing serum levels of TNF-α and AFP than either of both.

Table 3 shows the effect of the tested curcumin and/or naringin on the liver oxidative stress markers and antioxidant defense system of DMBA-administered rats. The treatment of these animals with mixture of curcumin and naringin produced a highly significant (p<0.05) increase of the glutathione (GSH) level as compared to the corresponding DMBA-administered group while the treatment with curcumin or naringin produced a non-significant (p>0.05) effect on GSH level.

Glutathione-S-transferase (GST) activity was significantly lowered in DMBA-administered rats. The treatment of DMBA-administered rats with curcumin and naringin normalized.

In contrast, hepatic lipid peroxidation (LPO) product was significantly (p<0.05) increased as a result of DMBA administration while the treatment of DMBA-administered rats with curcumin and/or naringin for 6 weeks significantly decreased the elevated values.
3.2. Histopathology:

Photomicrographs of the liver sections of normal animals showed normal histological structure of the central vein and surrounding hepatocytes at the two tested periods (Fig. 1A&B). Liver of animals treated with DMBA showed diffuse fatty changes in most of the hepatic parenchyma in association with focal areas of necrosis, dilatation and congestion in both central and portal veins and inflammatory cells infiltration in the portal areas after 2 weeks (Fig. 2). After 6 weeks, fatty changes were observed in most of the hepatocytes with other degenerative changes in association with congestion in the central vein (Fig. 3). Liver of animals administered DMBA and treated with curcumin showed diffuse fatty changes while other focal area of the parenchyma had other degenerative changes in association with dilatation in the portal vein after 2 weeks (Fig. 4). After 6 weeks, there was focal area of fatty change in the hepatocytes surrounding the central vein associated with congestion in the portal vein (Fig. 5). Liver of animals administered DMBA treated with naringin showed ballooning degeneration in the hepatocytes after 2 weeks (Fig. 6A) and few individual hepatocytes which showed fatty changes after 6 weeks (Fig. 6B). Liver of animals treated with mixture of curcumin and naringin appeared to markedly reduce DMBA-induced toxicity as evidenced by less dilatation and congestion in the central vein after 2 weeks (Fig. 7A) and fatty changes in few individual hepatocytes after 6 weeks (Fig. 7B).
**Table 1:** Effect of curcumin and/or naringin on serum AST, ALT, ALP and GGT activities in DMBA-administered rats at different periods of time.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>2 Weeks</th>
<th>6 Weeks</th>
<th>2 Weeks</th>
<th>6 Weeks</th>
<th>2 Weeks</th>
<th>6 weeks</th>
<th>2 weeks</th>
<th>6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AST (U/L)</td>
<td></td>
<td></td>
<td>ALT(U/L)</td>
<td></td>
<td>ALP(U/L)</td>
<td></td>
<td>GGT(U/L)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>28.28</td>
<td>± 0.53de</td>
<td>28.53</td>
<td>± 0.58de</td>
<td>28.82</td>
<td>± 2.08d</td>
<td>29.96</td>
<td>± 1.83d</td>
<td>219.08</td>
</tr>
<tr>
<td>DMBA</td>
<td>48.23</td>
<td>± 7.01b</td>
<td>65.48</td>
<td>± 4.44a</td>
<td>77.19</td>
<td>± 8.93ab</td>
<td>90.73</td>
<td>± 20.26a</td>
<td>444.26</td>
</tr>
<tr>
<td>DMBA + Curcumin</td>
<td>37.52</td>
<td>± 2.68ed</td>
<td>34.65</td>
<td>± 3.85cede</td>
<td>57.98</td>
<td>± 11.91bc</td>
<td>58.22</td>
<td>± 1.74bce</td>
<td>121.40</td>
</tr>
<tr>
<td>DMBA + Naringin</td>
<td>33.25</td>
<td>±3.54cede</td>
<td>43.15</td>
<td>± 2.34bc</td>
<td>45.49</td>
<td>± 3.91cd</td>
<td>36.85</td>
<td>± 5.31ed</td>
<td>187.56</td>
</tr>
<tr>
<td>DMBA + Curcumin + Naringin</td>
<td>25.82</td>
<td>± 3.74e</td>
<td>32.45</td>
<td>± 2.78de</td>
<td>34.53</td>
<td>± 4.76ed</td>
<td>39.36</td>
<td>± 3.26ed</td>
<td>199.31</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td>10.467</td>
<td></td>
<td>24.494</td>
<td></td>
<td>46.787</td>
<td></td>
<td>2.495</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD at 1%</td>
<td>14.096</td>
<td></td>
<td>32.986</td>
<td></td>
<td>63.009</td>
<td></td>
<td>3.361</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Data are expressed as Mean ± SE.
- Numbers of samples in each group is six.
- Values, which share the same superscript symbol, are not significantly different.
- Percentage changes were calculated by comparing DMBA administered group with normal control and DMBA administered groups treated with curcumin and/or naringin with DMBA-administered control groups.
Table 2: Effect of curcumin and/or naringin on serum Albumin, TNF-α and AFP levels in DMBA-administered rats at different periods of time.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Albumin (mg/dl)</th>
<th>TNF-α (pg/ml)</th>
<th>AFP (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 Weeks</td>
<td>6 Weeks</td>
<td>2 Weeks</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>4.46 ± 0.13a</td>
<td>4.47 ± 0.19a</td>
<td>14.56 ± 0.56f</td>
</tr>
<tr>
<td>DMBA</td>
<td></td>
<td>3.88 ± 0.20b</td>
<td>3.79 ± 0.04b</td>
<td>47.68 ± 4.80bc</td>
</tr>
<tr>
<td>DMBA + Curcumin</td>
<td></td>
<td>4.55 ± 0.08a</td>
<td>3.85 ± 0.03b</td>
<td>29.40 ± 0.25e</td>
</tr>
<tr>
<td>DMBA + Naringin</td>
<td></td>
<td>4.36 ± 0.02a</td>
<td>4.41 ± 0.20a</td>
<td>29.73 ± 2.40e</td>
</tr>
<tr>
<td>DMBA + Curcumin + Naringin</td>
<td></td>
<td>4.58 ± 0.17a</td>
<td>4.38 ± 0.19a</td>
<td>24.96 ± 1.22e</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td></td>
<td>0.403</td>
<td>7.259</td>
<td>0.150</td>
</tr>
<tr>
<td>LSD at 1%</td>
<td></td>
<td>0.542</td>
<td>9.776</td>
<td>0.202</td>
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</table>

Data are expressed as Mean ± SE. - Numbers of samples in each group is six. - Values, which share the same superscript symbol, are not significantly different. - percentage changes were calculated by comparing DMBA administered group with normal control and DMBA administered groups treated with curcumin and/or naringin with DMBA-administered control groups.
Table 3: Effect of curcumin and/or naringin on hepatic LPO, GSH and GST levels of DMBA-administered rats at different periods of time.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>LPO (nmole/mg)</th>
<th>GSH (nmole/mg)</th>
<th>GST (U/g)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>2 Weeks</td>
<td>6 Weeks</td>
<td>2 Weeks</td>
</tr>
<tr>
<td><strong>Normal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>32.53± 1.93e</td>
<td>33.42± 2.43de</td>
<td>27.51± 2.53bcd</td>
</tr>
<tr>
<td><strong>DMBA</strong></td>
<td></td>
<td>45.01± 2.54bcd</td>
<td>76.23± 8.53a</td>
<td>13.89± 2.31f</td>
</tr>
<tr>
<td><strong>DMBA + Curcumin</strong></td>
<td></td>
<td>39.49± 4.48cde</td>
<td>40.86± 4.51cde</td>
<td>15.20± 0.66f</td>
</tr>
<tr>
<td><strong>DMBA + Naringin</strong></td>
<td></td>
<td>32.97± 2.32de</td>
<td>50.84± 5.16bc</td>
<td>18.51± 1.35er</td>
</tr>
<tr>
<td><strong>DMBA + Curcumin + Naringin</strong></td>
<td></td>
<td>43.99± 2.72bcde</td>
<td>53.24± 3.78b</td>
<td>22.81± 2.29de</td>
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<td><strong>LSD at 5%</strong></td>
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<td>12.360</td>
<td>6.282</td>
<td>34.283</td>
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<td><strong>LSD at 1%</strong></td>
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<td>16.646</td>
<td>8.461</td>
<td>46.164</td>
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Data are expressed as Mean ± SE. - Numbers of samples in each group is six.
- Values, which share the same superscript symbol, are not significantly different.
- percentage changes were calculated by comparing DMBA-administered group with normal control and DMBA-administered groups treated with curcumin and/or naringin with DMBA-administered control groups.
Figs. 1: Photomicrographs of liver sections of normal control rats showing normal histological structure of the central vein (cv) and surrounding hepatocytes (h) after 2 (Fig. 1A, X400) and 6 weeks (Fig. 1B, X400).

Figs 2: Photomicrographs of liver sections of rats -administered DMBA after 2 weeks showing diffuse fatty changes (f) in most of the hepatic parenchyma while other focal areas showed necrosis (n) in association with dilatation and congestion in both central (cv) and portal veins (pv) as well as inflammatory mononuclear (m) leukocytes infiltration in the portal area (Fig. 2A, X100; Fig. 2B, X400).

Fig. 3: Photomicrographs of liver sections of rats -administered DMBA after 6 weeks showing fatty change (f) in most of the hepatocytes associated with degenerative changes and congestion in the central vein (cv) (X400).
Fig. 4: Photomicrograph of liver section of rat -administered DMBA and treated with curcumin for 2 weeks showing diffuse fatty changes (f) associated with focal areas of degenerative changes, necrosis and dilatation in the portal vein (pv) (X100).

Fig. 5: Photomicrograph of liver section of rat -administered DMBA and treated with curcumin for 6 weeks showing focal areas of fatty changes (f) in the hepatocytes surrounding the central vein (cv) (X100).

Figs 6: Photomicrographs of liver sections of rats -administered DMBA and treated with naringin showing ballooning degeneration (d) in the hepatocytes after 2 weeks (Fig. 6A, X400) and few individual hepatocytes showed fatty changes (f) after 6 weeks (Fig. 6B, X400).

Figs 7: Photomicrographs of liver sections of rats -administered DMBA and treated with a mixture of curcumin and naringin showing marked reduction in DMBA-induced toxicity as evidenced by less
dilatation and congestion in the central vein (cv) after 2 weeks (Fig. 7A, X100) and fatty changes (f) in few individual hepatocytes after 6 weeks (Fig. 7B, X400).

Discussion

The metabolic activation and detoxification of DMBA in vivo are known to occur primarily in the liver and also in a variety of other organs including the mammary gland. The metabolism of DMBA in the liver often quantitatively predominates over organ specific metabolism (Nandakumar et al., 2011).

In the present study, serum AST, ALT, ALP and GGT activities were significantly increased as a result of administration of DMBA at the 2nd and 6th weeks while serum albumin level was significantly decreased reflecting impairment in liver function and liver injury. These results are in agreement with those obtained by many authors (Harmeet et al., 2011; Pandi et al., 2011; Bedi and Sinha Priyanka, 2012). These DMBA-induced changes were significantly prevented as a result of administration curcumin and/or naringin for 2 and 6 weeks. These improvement effects of curcumin and naringin on liver function are reported by many other investigators. Yousef et al. (2008) revealed that administration of curcumin exerted similar hepatoprotection following arsenic intoxication in rats. It was also reported that turmeric and its active principles the curcuminoids can exert protection either directly by shielding the biomolecule or indirectly by stimulating the natural detoxification and defense mechanisms of the body (Ross, 2003 and Aggarwal et al., 2007). On the other hand, Jeon et al. (2004) speculated that administration of naringin may stabilize the hepatic cellular membrane and protect the hepatocytes against toxic effects of nickel leading to decrease in the leakage of the enzymes into blood stream. They attributed this protective effect to the antioxidant property of naringin. In the same regard, Thangavel et al. (2012) demonstrated that treatment with naringin attenuated the increased activities of these enzymes (AST, ALT and ALP) in N-nitrosodiethylamine-induced liver carcinogenesis.

Based on these results and evidences, the increase in ALT, AST, GGT, ALP activities and decreased in albumin level reflects the observed derangements in the histological architecture in DMBA-administered rats. The liver histological deleterious effect of DMBA include diffuse fatty changes, focal areas of necrosis, inflammatory cells infiltration, and dilatation and congestion of central and portal veins. These results are in accordance with Nandakumar et al. (2011) and Rengarajan et al. (2012) who indicated that DMBA-induced cancer bearing animals were showed loss of architecture with enlarged sinusoids and tendency to spread by intrahepatic veins with significant abnormalities in portal vessels.
In the present study, the histolopathological changes of liver were remarkably amended due to curcumin treatment. Our results go parallel with Seehofer et al. (2009) who observed that curcumin treated groups showed only minimal fatty changes and a low hepatocyte density due to hepatocellular hypertrophy. Also, these results in concordance with Naik et al. (2011) who indicated that the curcumin showed a moderate to marked diffused granular degeneration and moderate degree of centrilobular fatty infiltration with moderate leucocytic infiltration.

Similar to curcumin, the histolopathological changes of liver were detectably ameliorated due to naringin treatment. These results are in accordance with Thangavel et al. (2012) who observed that rats treated with naringin and N-nitrosodiethylamine exhibited few neoplastically transformed cells and hepatocytes maintaining near normal architecture. Also our results are concomitant with Pari and Amudha (2011) who observed that liver of nickel-intoxicated male Wistar rats treated with naringin show normal hepatocytes with mild portal inflammation and with Pu et al. (2012) who found that treatment with naringin attenuated lipid deposition and inflammation of liver in high fat-fed rats. They attributed to these ameliorations to the antioxidant and chelating ability of naringin, which significantly reduced the oxidative threat leading to reduction of pathological changes and restoration of normal physiological functions.

The histopathological changes of liver treated with mixture of curcumin and naringin appeared to significantly reduce DMBA-induced toxicity as evidenced by less dilatation and congestion in the central vein after 2 weeks and fatty changes in few individual hepatocytes after 6 weeks. Thus, it can be suggested that curcumin and naringin may act synergistically to produce more potent ameliorative effects.

In our study, the pro-inflammatory cytokine, TNF-α, and tumor marker, AFP, levels exhibited a marked increase in serum of DMBA-administered rats as compared to the normal ones. The treatment of DMBA-administration rats with curcumin and/or naringin caused marked amelioration of these estimated variables. These results agree well with Hayashi and Sakai (2011), Luo et al. (2012) and Chen et al. (2013). TNF-α is a pleiotropic inflammatory cytokine involved in cell death/apoptosis (Hayashi and Sakai, 2011). Thus, the decrease in serum TNF-α level in DMBA-administered rats as a result of treatment with curcumin and/or naringin in association with the decrease or absence of inflammatory leucocytic infiltration in the liver parenchyma reflects the anti-inflammatory role of these plant constituents. Alpha fetoprotein is a fetal specific glycoprotein which falls rapidly after birth, high level of alpha
fetoprotein is suspicious of hepatocellular carcinoma but may be elevated in chronic viral hepatitis (Patil et al., 2013). Its decrease in DMBA-administered rats treated with curcumin and/or naringin may provide evidence for decreased probability for hepatocellular carcinoma as compared with DMBA-administered control rats which showed profound elevation of serum AFP.

Oxidative damage occurs to cells in vivo and in vitro from exposure to free radicals generated by exogenous agents (e.g., radiation, chemicals, hyperoxia) and endogenous processes such as normal cellular metabolism. An imbalance between antioxidants and ROS results in oxidative stress, leading to cellular damage (Moreira da Silva et al., 2010). Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite (Moreira da Silva et al., 2010).

In the present study, the treatment of the DMBA-administered animals with curcumin and/or naringin potentially decreased the elevated liver lipid peroxidation, a major contributor to the loss of cell function under oxidative stress conditions (Nandakumar et al., 2011) and enhanced the antioxidant defense system by increasing the hepatic glutathione content and glutathione-S-transferase activity. Based on these results, it can be suggested that the improvement effects of curcumin and naringin on the impaired liver function and histological integrity of DMBA treated rats may be mediated at least in part by suppressing the oxidative stress and alleviation of the anti-oxidant defense system. The capacity of curcumin against the oxidative stress might be due to its intrinsic structure which could donate protons from both the phenolic hydroxyl group and the methylene group between the beta-diketone (Barzegar and Moosavi-Movahedi, 2011). As reported by Amudha and Pari (2011), the antioxidant efficacy of naringin may be due to its capability to react with free radicals or with highly reactive products of lipid peroxidation as well as enhancement of tissue thiol pools may be responsible for the reduction of oxidative stress.

Taken the previous findings and suggestion together, it can be concluded that the curcumin and/or naringin in have a potent preventive effects against DMBA-induced deleterious effects on the liver function and histological integrity. These preventive effects may be mediated at least in part via suppressing the oxidative stress, attenuating inflammation and improving the antioxidant defense system. The more potent effect of mixture of curcumin and naringin than either of both on liver function, antioxidant defense system as well as on histological architecture may provide evidence for the synergistic action of both plant constituents.
5. References:


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