Anti-carcinogenic potential of *Euphorbia neriifolia* leaves and isolated flavonoid against N-Nitrosodiethylamine-induced renal carcinogenesis in mice

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Anti-carcinogenic potential of hydro-ethanolic extract of *Euphorbia neriifolia* (EN) leaves and an isolated flavonoid (ENF) was investigated against N-Nitrosodiethylamine (DENA)-induced renal carcinogenesis in mice. Experimental mice were pretreated with 150 and 400 mg/kg body wt of EN, 0.5% and 1% mg/kg body wt of butylated hydroxylanisole (BHA) as a standard antioxidant and 50 mg/kg body wt of ENF for 21 days prior to the administration of a single dose of 50 mg/kg body wt of DENA. Levels of renal markers (urea and creatinine), xenobiotic metabolic enzymes (Cyt P450 and Cyt b5), lipid peroxidation (LPO), antioxidants (SOD, CAT, GST and GSH) and other biochemical parameters — AST, ALT, ALP, total protein (TP), and total cholesterol (TC) were measured to determine the renal carcinogenesis caused by DENA. DENA administration significantly (p<0.001) decreased the body weight and increased the tissue weight. It significantly (p<0.001) enhanced the levels of Cyt P450, Cyt b5 and LPO and decreased the levels of SOD, CAT, GST and GSH content. The activities of AST, ALT and ALP and the TP content and renal markers were also significantly decreased (p<0.001), while TC level was markedly increased after DENA administration, as compared with the normal control group (p<0.001). Pretreatment with EN and ENF counteracted DENA-induced oxidative stress (LPO) and exerted its protective effects by restoring the levels of antioxidants (SOD, CAT, GST and GSH), biochemical parameters (AST, ALT, ALP, TP and TC), renal markers (urea and creatinine) and xenobiotic enzymes (Cyt P450 and Cyt b5) in renal tissue. In conclusion, the present study showed significant anti-carcinogenic potential of the hydro-ethanolic extract of *E. neriifolia* and ENF against DENA-induced renal carcinogenicity.

**Keywords:** N-Nitrosodiethylamine, *Euphorbia neriifolia*, Antioxidant, Renal carcinogenesis, Flavonoid, BHA

Kidney cancer is the third most common malignancy of the genitourinary system in the world and accounts for 2-3% of all cancers in men and 1-2% in women; 130,000 new cases and 63,000 deaths occur annually from the disease. Certain toxic chemicals and medicines are reported to cause renal damage. N-Nitrosodiethylamine (DENA), an important environmental carcinogen among the nitrosamines is considered to be a human carcinoma based on evidences of carcinogenicity in experimental animals. DENA has been suggested to cause oxidative stress and cellular injury, due to involvement of free radicals. It is found in drinking water, foodstuffs (milk and meat products), alcoholic beverages, soft drinks and a few varieties of vegetables like soybeans. Lipid peroxidation and associated membrane damage are key features of DENA-induced carcinogenesis. In modern medicine, beneficial effects of synthetic drugs are well-documented; however, they often are associated with some toxic effects, such as carcinogenicity. Thus there is a need for more effective antioxidants of natural origin that have significant scavenging properties and are less toxic and inherently safer than synthetic antioxidants.

*Euphorbia neriifolia* Linn. (Euphorbiaceae) is a large succulent shrub or small tree up to 15-20 ft in height with stipular thorns and known as “Sehund or Siju” in Hindi and Thohar in Rajasthan, is found throughout the Deccan Peninsula of India. *E. neriifolia* (leaves, stem and latex) contains wide range of active ingredients, such as flavonoids, alkaloids, tannins, triterpenoids, etc.
Glut-5(10)-en-1-one, taraxerol and $-amyrin, neriifolione], tetracyclic triterpene (nerifoliene and euphol), triterpenoidal saponins etc. which are useful in abdominal troubles, bronchitis, tumors, loss of consciousness, delirium, leucoderma, piles, inflammation, anaemia, ulcers, fever and enlargement of spleen.

Earlier reports described the antioxidant, free radical scavenging, haematological, biochemical as well as analgesic activities of E. neriifolia leaves. In the present study, we have investigated the effect of 21 days treatment of hydro-ethanolic extract of leaves of E. neriifolia (EN) and an isolated flavonoid [2-(3,4-dihydroxy-5-methoxy-phenyl)-3,5-dihydroxy-6,7-dimethoxycromen-4-one] designated as ENF (Fig. 1) against DENA-induced renal carcinogenicity in mice.

Materials and Methods

Chemicals

N-Nitrosodiethylamine (DENA) was purchased from Sigma Chemical Co., USA. All other chemicals used were of analytical grade and purchased from reliable firms (SRL and Qualigens).

Preparation of extract

Euphorbia neriifolia leaves were collected from medicinal garden of Banasthali University, Banasthali (Rajasthan, India) and nearby areas of Banasthali (Latitude N-26°24’14.8414”; Longitude E-73°52’9.7194”) in the month of September 2009 and taxonomically identified by botanist of Bioscience and Biotechnology Department, Banasthali University, Banasthali, Rajasthan, India. A voucher specimen was deposited in the herbarium of the department. Shade-dried leaves were powdered, soxhlet extracted with 70% (v/v) ethanol and concentrated to dryness under reduced pressure at 60 ± 1°C. After drying in hot air oven (40-45°C), the sample was put in an air tight container and stored in refrigerator at 5°C. The sample was designated as hydro-ethanolic extract of EN leaves and was used whenever required to assess nephro-protective activity.

Extraction and isolation of flavonoids

Dried leaves of E. neriifolia (250 g) were extracted successively with pet-ether, benzene, chloroform, ethyl acetate and ethanol and finally macerated with distilled water (non-polar to polar) to obtain respective extracts. Presence of flavonoids was determined by the method of Harborne using quercetin and rutin as standards. All the extracts showed presence of flavonoid compounds with TLC and HPTLC technique using standard mobile phases. n-Butanol: acetic acid: water (BAW, 4: 1: 5; 2: 2: 6) was found to be most appropriate solvent system for separation of flavonoids. Out of six extracts, dark-brown sticky semi-solid ethanolic extract (48.9 g) contained bulk of flavonoids and was used for further chromatographic separation. Iodine (I$_2$) and ammonia (NH$_3$) vapours were used as a developer.

Ethanolic extract was concentrated and chromatographed on silica plates using n-butanol: acetic acid: water (2: 2: 6) as mobile phase. Three spots were resolved and designated as IF$_1$, IF$_2$ and IF$_3$ having R$_f$ values of 0.60, 0.79 and 0.90, respectively. The R$_f$ of IF$_2$ coincided with standard quercetin (R$_f$: 0.79). Maximum yield was obtained in IF$_2$ (13.4%), which was crystallized using ethanol giving solid pale yellow crystals, soluble in water and organic solvents. It showed positive results (alkaline, lead and ammonia tests) for flavonoid and negative for steroids, terpenoids and alkaloids. IR spectrum of IF$_2$ in KBr pellet confirmed the presence of hydroxyl (-OH) group with H-bonded primary alcohol in flavonoids. The flavonoid was characterized by using $^1$H NMR and mass spectroscopy as 2-(3,4-dihydroxy-5-methoxy-phenyl)-3,5-dihydroxy-6,7-dimethoxycromen-4-one and designated as E. neriifolia flavonoid (ENF; Fig. 1) and was used to assess nephro-protective activity.

Experimental animals and treatment regimen

Healthy male Swiss albino mice (Mus musculus L.) procured from C.C.S. Haryana Agricultural University, Hissar (Haryana, India) were housed under standard laboratory conditions of temperature (22 ± 3°C), relative humidity (50 ± 15%) and photoperiod (12:12 h L:D cycle). Animals had free access to standard food pellet diet (Hindustan Lever Ltd., India) and tap water ad libitum. The experiments were carried out in accordance with the guidelines.
given by Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg. No. IAEc/814, dated. 23. 01. 2010).

After two weeks of acclimatization, mice were randomly divided into twelve groups of 6 mice each. The groups for each parameter were as follows: Group I (normal control, NC); Group II (carcinogen control, CC, received distilled water for 21 days prior to a single dose of DENA, 50 mg/kg body wt, p.o); Groups III and IV (E. neriifolia low dose, ENL 150 mg/kg body wt/day and E. neriifolia high dose, ENH 400 mg/kg body wt/day, p.o for 21 days); Groups V and VI (BHA low dose, BHAL 0.5% and BHA high dose, BAH 1% mg/kg body wt/day, p.o for 21 days, dissolved in 0.5% acetone, standard treated group); and Group VII (ENF 50 mg/kg body wt/day; p.o, for 21 days, dissolved in distilled H2O). The groups VIII-XII were first pre-treated with EN, BHA (low and high dose) and ENF for 21 days and on day 22 DENA was administered and left for 10 days. Body weight was measured at regular intervals during experimental period of 31 days. The dose for DENA (Sigma-N0258-Material Safety Data Sheet, 2003), EN, BHA and ENF were selected on the basis of LD50 calculated in our own laboratory and other published reports.

After completion of treatment period, the animals of all groups were euthanized by cervical dislocation. Kidneys were excised immediately, washed, cleaned and rinsed with ice-cold normal saline solution (0.9% NaCl), pH 7.4, until bleached of all the blood and blotted dry on filter paper sheets to remove blood. The wet weight of the organ was noted only after drying the tissue. Tissue homogenate was prepared in ice-cold 0.1 M sodium phosphate buffer (pH-7.4) at 1-4°C (10% homogenate w/v). Homogenate was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was kept frozen (-80°C) until assayed.

Estimation of kidney markers
Kidney markers (urea and creatinine) were measured using Erba, Mannheim, Transasia Kits.

Biochemical parameters
Aspartate aminotransferase (AST)20, alanine aminotransferase (ALT)20, alkaline phosphatase (ALP)21, total protein content (TPC)22 and total cholesterol (TC)23 levels were also estimated in renal homogenate.

Determination of xenobiotic enzymes
Phase I xenobiotic enzymes i.e. cytochrome P450 and cytochrome b5 contents were assayed in the renal homogenate by the method of Omura and Sato24 using an absorption coefficient of 91 and 185 cm2/mmol.

Cellular metabolic parameters
Lipid peroxidation in renal tissue was determined by measuring the accumulation of thiobarbituric acid-reactive substance and expressed as malondialdehyde (MDA) content25. Kidney metabolic enzymatic antioxidants — superoxide dismutase (SOD)26, catalase (CAT)27, glutathione-S-transferase (GST)28 and non-enzymatic antioxidant (GSH)29 were also estimated.

Statistical analysis
Results were expressed as mean ± SEM. The data were subjected to one-way analysis of variance (ANOVA), followed by Tukey’s post-hoc multiple comparison test by fixing significant values as p<0.001, p<0.01 and p<0.005 using the S.P.S.S. (version 16.0) program.

Results and Discussion
In the present study, in vivo preventive effect of 21 days treatment of hydro-ethanolic extract and isolated flavonoid of E. neriifolia leaves was investigated against renal carcinogenesis induced by DENA.

Effect on body weight
Significant (p<0.01) decrease in body weight was observed in carcinogen control (CC) group II, as compared to non-treated group. Similar results have also been observed in an earlier study30. E. neriifolia extract (groups III and IV) caused dose-dependent increase in body weight, as compared to NC group I (Table 1). This finding was in agreement with the earlier report7. Significant decrease in body weight was also recorded in mice treated with BHA (0.5% and 1%). Pre-treatment with EN extract (groups VIII and IX) and ENF (group XII) before intoxicated with DENA improved the loss in body weight, suggesting a protective effect of E. neriifolia (150 and 400 mg/kg body wt) and ENF in these animals. ENH and ENF were more effective as compared to ENL and BHA (at both doses).

Effect on organ weight
Organ body weight ratios are normally investigated to determine whether the treatment has any effect on size and weight of the vital organs comparative to total body weight. Compared to control, absolute weight of renal tissue was significantly (p<0.05) increased in CC (group II). Administration of EN and
A low urea and creatinine, as compared to normal control significantly (p<0.001; p<0.01) altered the levels of nephron structural integrity. Creatinine levels might be attributed to the damage due to renal injury. The depletion in tissue urea and creatinine (markers of kidney function) in kidney, indicating (p<0.001) decreased the levels of urea and creatinine, which was evidenced by biochemical measurements and was in agreement with the previous reports. DENA-induced nephrotoxicity was evidenced by alterations in urea and creatinine levels, as compared to NC group (Table 1). Pre-administration of EN, BHA (at both doses) and ENF against DENA-induced renal carcinogenicity in mice.

**Table 1—Effect of EN, BHA (at both doses) and ENF on organ weight, body weight and on urea and creatinine against DENA-induced renal carcinogenicity in mice**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Absolute organ wt (g)</th>
<th>Relative organ wt (%)</th>
<th>Final body wt (g)</th>
<th>Urea (mg/100g)</th>
<th>Creatinine (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC (I)</td>
<td>0.45 ± 0.02</td>
<td>1.40</td>
<td>32.05 ± 2.21</td>
<td>33.45 ± 0.12</td>
<td>1.42 ± 0.05</td>
</tr>
<tr>
<td>CC (II)</td>
<td>0.62 ± 0.06</td>
<td>3.34</td>
<td>18.58 ± 2.54</td>
<td>22.81 ± 0.21</td>
<td>0.82 ± 0.08</td>
</tr>
<tr>
<td>ENL (III)</td>
<td>0.47 ± 0.05</td>
<td>1.39</td>
<td>33.83 ± 2.45</td>
<td>36.18 ± 0.14</td>
<td>1.51 ± 0.05</td>
</tr>
<tr>
<td>ENH (IV)</td>
<td>0.49 ± 0.06</td>
<td>1.41</td>
<td>34.86 ± 3.06</td>
<td>39.63 ± 0.12</td>
<td>1.61 ± 0.03</td>
</tr>
<tr>
<td>BHAL (V)</td>
<td>0.45 ± 0.03</td>
<td>1.83</td>
<td>24.54 ± 3.58</td>
<td>34.22 ± 0.25</td>
<td>1.46 ± 0.04</td>
</tr>
<tr>
<td>BHAH (VI)</td>
<td>0.46 ± 0.06</td>
<td>2.07</td>
<td>22.18 ± 3.10</td>
<td>34.84 ± 0.13</td>
<td>1.48 ± 0.05</td>
</tr>
<tr>
<td>ENF (VII)</td>
<td>0.49 ± 0.08</td>
<td>1.42</td>
<td>34.51 ± 1.50</td>
<td>38.72 ± 0.18</td>
<td>1.54 ± 0.06</td>
</tr>
<tr>
<td>ENL + CC (VIII)</td>
<td>0.48 ± 0.04</td>
<td>1.69</td>
<td>28.33 ± 2.34</td>
<td>28.52 ± 0.24</td>
<td>1.18 ± 0.10</td>
</tr>
<tr>
<td>ENH + CC (IX)</td>
<td>0.44 ± 0.07</td>
<td>1.45</td>
<td>31.67 ± 2.98</td>
<td>31.61 ± 0.14</td>
<td>1.38 ± 0.06</td>
</tr>
<tr>
<td>BHAL + CC (X)</td>
<td>0.58 ± 0.03</td>
<td>2.85</td>
<td>20.32 ± 3.44</td>
<td>24.35 ± 0.12</td>
<td>0.91 ± 0.12</td>
</tr>
<tr>
<td>BHAH + CC (XI)</td>
<td>0.56 ± 0.05</td>
<td>2.39</td>
<td>23.47 ± 3.06</td>
<td>25.72 ± 0.23</td>
<td>1.01 ± 0.07</td>
</tr>
<tr>
<td>ENF + CC (XII)</td>
<td>0.48 ± 0.03</td>
<td>1.59</td>
<td>30.15 ± 1.95</td>
<td>29.56 ± 0.16</td>
<td>1.26 ± 0.05</td>
</tr>
</tbody>
</table>

Significance change is calculated as **p<0.001 compared to NC group; *p<0.01 compared to NC group; 3p<0.05 compared to NC group** and **p<0.001 compared to CC mice; *p<0.01 compared to CC mice, †p<0.05 compared to CC mice Cytochrome P450 and b5: n mole/mg. NC: Normal control group; CC: Cancer/carcinogenic control group (DENA); ENL: EN low dose; ENH: EN high dose; BHAL: BHA low dose; BHAH: BHA high dose; ENF: E. neriifolia flavonoid.**

BHA (at both doses) and ENF alone caused insignificant alterations in absolute organ weight in comparison to NC group I. Pre-treatment with ENL (p<0.05), ENH (p<0.01) and ENF (p<0.01) prior to intoxication with DENA markedly reduced the mean weight of kidney as compared to the CC (group II), indicating the protective effect of extract on carcinogen exposure. Similarly, pre-treatment with BHAH prior to intoxication with DENA significantly (p<0.01) reduced the tissue weight, whereas BHAL insignificantly altered the relative weight of kidney, as compared to CC (group II). In comparison to EN extract and ENF, BHA was less effective and did not modulate the organ weight towards normal (Table 1).

The pathological changes induced by DENA were monitored by determining the levels of various biochemical and oxidative stress renal markers. DENA-induced nephrotoxicity was evidenced by biochemical measurements and was in agreement with the previous reports.

**Effect on kidney markers**

DENA administration (group II) significantly (p<0.001) decreased the levels of urea and creatinine (markers of kidney function) in kidney, indicating renal injury. The depletion in tissue urea and creatinine levels might be attributed to the damage of nephron structural integrity. Administration of EN extract and BHA (at both doses) and ENF significantly (p<0.001; p<0.01) altered the levels of urea and creatinine, as compared to normal control (group I). Pre-administration of EN, BHA (at both doses; groups VIII to XI) and ENF (group XII) prior to the administration of DENA significantly (p<0.001; p<0.01) normalized the levels of urea and creatinine (Table 1). EN at higher dose and ENF showed significant alteration in urea and creatinine levels, as compared to the lower dose of EN extract and BHA (at both doses). This effect might be due to the antioxidant properties of EN.

**Effect on biochemical parameters**

DENA administration produced a significant (p<0.001) decrease in AST, ALT and ALP levels, as compared to NC mice (group I). The decrease could be attributed to the release of these enzymes from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage. DENA (50 mg/kg body wt) caused severe distortion of cytoarchitecture of renal tissue and induced carcinogenicity which was confirmed by histological examination (data not shown). Histopathological findings revealed the degenerative changes in glomerulus, renal tubules and vacuolization of cells.

AST, ALT and ALP levels in kidney homogenate were significantly (p<0.001; p<0.01) increased by the administration of EN, BHA (at both doses) and ENF (vs. untreated control mice). Pre-treatment with EN, BHA (at both doses; groups VIII to XI) and ENF (group XII) prior to DENA administration significantly (p<0.001) increased the levels of AST, ALT and ALP, as compared with CC (group II; Table 2).
of renal cells accelerates the regeneration process and production of protective activity, as stimulation of protein synthesis (at both doses; groups VIII and IX) indicated renal intoxication with DENA significantly (p<0.001; p<0.01) increased level of TP by EN augmented the protein level, as compared to CC group (Table 2). Increased level of TP in renal tissue in comparison to NC group. A significant (p<0.001; p<0.01) increase due to the inhibition of bile acids synthesis from cholesterol, which is synthesized in kidney. A significant decrease (p<0.001; p<0.01) in TC content was observed in groups III to VII, when EN (at both doses; groups VIII to XI) and ENF (group XII) before administration of DENA restored the activities of these enzymes to near normal values. These findings confirmed that EN (at both doses) and ENF preserved the structural integrity of the organs from the toxic effects of DENA.

Total cholesterol (TC) content in DENA-administered mice was significantly (p<0.001) increased in comparison to NC group. This might be due to the inhibition of bile acids synthesis from cholesterol, which is synthesized in kidney. A significant decrease (p<0.001; p<0.01) in TC content was observed in groups III to VII, when compared with NC group. Pre-treatment with EN, BHA (at both doses; groups VIII to XI) and ENF (group XII) before intoxication with DENA significantly (p<0.001; p<0.01) decreased TC content, as compared with carcinogen control (Table 2).

DENA administration significantly (p<0.001) decreased the TP content in renal tissue in comparison to NC group. A significant (p<0.001; p<0.01) increase in TP content was observed on treatment with EN at the dose of 150 and 400 mg/kg body wt, BHA (0.5% and 1%) and ENF (group VII) when compared with normal treated group I. Administration of EN, BHA (at both doses) and ENF before intoxication with DENA significantly (p<0.001; p<0.01) augmented the protein level, as compared to CC group (Table 2). Increased level of TP by EN (at both doses; groups VIII and IX) indicated renal protective activity, as stimulation of protein synthesis accelerates the regeneration process and production of renal cells.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
<th>TC</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC (I)</td>
<td>38.55 ± 0.07</td>
<td>34.36 ± 0.10</td>
<td>59.37 ± 0.13</td>
<td>90.96 ± 0.09</td>
<td>6.02 ± 0.06</td>
</tr>
<tr>
<td>CC (II)</td>
<td>16.50 ± 0.09</td>
<td>14.49 ± 0.18</td>
<td>28.34 ± 0.29</td>
<td>138.14 ± 0.11</td>
<td>4.08 ± 0.09</td>
</tr>
<tr>
<td>ENL (III)</td>
<td>43.22 ± 0.34</td>
<td>37.34 ± 0.30</td>
<td>62.22 ± 0.31</td>
<td>81.82 ± 0.34</td>
<td>7.20 ± 0.07</td>
</tr>
<tr>
<td>ENH (IV)</td>
<td>47.26 ± 0.09</td>
<td>39.45 ± 0.14</td>
<td>66.45 ± 0.37</td>
<td>75.33 ± 0.14</td>
<td>7.93 ± 0.11</td>
</tr>
<tr>
<td>BHAL (V)</td>
<td>39.21 ± 0.38</td>
<td>34.77 ± 0.28</td>
<td>59.83 ± 0.12</td>
<td>88.63 ± 0.12</td>
<td>6.43 ± 0.08</td>
</tr>
<tr>
<td>BHAH (VI)</td>
<td>41.32 ± 0.13</td>
<td>36.21 ± 0.42</td>
<td>61.15 ± 0.17</td>
<td>85.26 ± 0.08</td>
<td>6.97 ± 0.22</td>
</tr>
<tr>
<td>ENF (VII)</td>
<td>44.27 ± 0.08</td>
<td>37.82 ± 0.26</td>
<td>64.09 ± 0.29</td>
<td>79.73 ± 0.07</td>
<td>7.67 ± 0.08</td>
</tr>
<tr>
<td>ENL + CC (VIII)</td>
<td>29.31 ± 0.08</td>
<td>26.22 ± 0.31</td>
<td>45.53 ± 0.32</td>
<td>114.06 ± 0.21</td>
<td>5.37 ± 0.05</td>
</tr>
<tr>
<td>ENH + CC (IX)</td>
<td>34.22 ± 0.16</td>
<td>31.24 ± 0.12</td>
<td>53.62 ± 0.15</td>
<td>98.34 ± 0.27</td>
<td>5.88 ± 0.14</td>
</tr>
<tr>
<td>BHAL + CC (X)</td>
<td>21.03 ± 0.36</td>
<td>18.52 ± 0.14</td>
<td>34.21 ± 0.24</td>
<td>132.05 ± 0.23</td>
<td>4.62 ± 0.09</td>
</tr>
<tr>
<td>BHAH + CC (XI)</td>
<td>24.43 ± 0.25</td>
<td>21.11 ± 0.31</td>
<td>41.21 ± 0.45</td>
<td>124.06 ± 0.26</td>
<td>4.92 ± 0.12</td>
</tr>
<tr>
<td>ENF + CC (XII)</td>
<td>29.53 ± 0.13</td>
<td>27.23 ± 0.25</td>
<td>47.33 ± 0.24</td>
<td>112.48 ± 0.08</td>
<td>5.55 ± 0.18</td>
</tr>
</tbody>
</table>

Table 2—Effect of EN, BHA (at both doses) and ENF on renal biochemical parameters in DENA-induced renal carcinogenicity in mice

Significance change is calculated as *p<0.001 compared to NC group; **p<0.01 compared to NC group; and ***p<0.001 compared to CC mice; ^p<0.01 compared to CC mice. AST and ALT: IU/L; ALP: μmole PNP min⁻¹ g⁻¹ tissue; TC: mg/g; TP: mg/m.
findings suggested that EN and ENF acted as bifunctional inducer or “blocking agent”, as it induced both phase-I system enzymes (Cyt P450 and b5) that furnished the balance of xenobiotic metabolism towards detoxification and augmented the sequential reduction of xenobiotic substrates preparing them for phase-II metabolism.

**Cellular metabolic parameters**

Lipid peroxidation (LPO) plays an important role in carcinogenesis. Free radicals react with lipids and cause peroxidation, which results in formation of several toxic products, such as malondialdehyde (MDA), F2-isoprostanes, 4-hydroxynonenal, H2O2 and hydroxyl radicals. These products cause serious damage to cell membranes, inhibit several important enzymes, reduce cellular function and cause cell death.

In present study, DENA administration significantly (p<0.001) increased the TBARS level. EN extract (at both doses, groups III and IV; p<0.001), BHA (groups V and VI; p=0.01; p<0.01) and ENF (p<0.001) significantly decreased the TBARS level, as compared to NC animals (group I). Administration of EN, BHA and ENF before being challenged with DENA (groups VIII to XII) also showed a significant (p<0.001; p<0.01) decrease in TBARS level, as compared to CC group (Table 4). ENH (group IX) treatment was found to be more effective in comparison to ENF (group XII) and BHA (at both doses; groups X and XI). This protective effect might be due to the presence of wide range of polyphenols and active ingredients in the EN extract. These active ingredients might be counteracting this mechanism through a cytoprotective action of the glomerular mesangial cells, exerted by a restriction on apoptosis. ENF also modulated the peroxidation level towards normal, possibly due to antioxidant properties of flavonoids, which protect tissues against oxygen free radicals and LPO. Flavonoids may help in the prevention of cancer and chronic inflammation and might affect the bioenergetics in metabolic systems and may inhibit several oxidative coupling reactions by scavenging the free radicals present in the metabolic systems.

SOD, CAT, GST and GSH levels were significantly (p<0.001) decreased in CC group, as compared to NC group. SOD and CAT (metallo-protein enzymes) activities were decreased due to the generation of superoxide radicals in damaged kidney of CC group. Significant decrease in the activity of GST in CC (group II) might be due to the decreased expression of these antioxidants and enhanced LPO level during renal cellular damage.

EN treatment (at both doses) significantly (p<0.001; p<0.05) increased the SOD and GST levels and insignificantly increased the CAT level. GSH content increased insignificantly at lower dose (group III) and at higher dose (group IV), the significant (p<0.05) increase was noticed in comparison to NC animals (group I). BHA significantly (p<0.001) increased the SOD level at higher dose and insignificantly increased the SOD level at lower dose, as compared to NC group. Significant (p<0.001) increase in GST activity along with insignificant increase in CAT and GSH levels were observed in groups V and VI animals, when compared to NC mice.
high dose was most effective in amelioration of drug metabolizing enzymes in renal tissue followed by ENF>ENL>BHAH>BHAL.

The possible mechanism(s) of protection against DENA-induced renal toxicity by EN might be due to significant modulation of phase I (Cyt P450 and Cyt b5) and -II enzymes (SOD, CAT and GST) and antioxidative parameters. Thus, modulating effects on these parameters might have resulted in enhanced carcinogen detoxification. The attenuating activity of EN against the DENA-induced carcinogenicity might be due to the presence of flavonoids and phenolic compounds in EN. Flavonoids have been reported as potentially useful exogenous agents in protecting the organs and tissues of the body against free radical-induced damage. They, in particular quercetin, have been reported to inhibit xenobiotic-induced nephrotoxicity in experimental animals, due to their potent anti-oxidant, free radical scavenging and metal chelating abilities.

In conclusion, the present study demonstrated the significant anti-carcinogenic potential of the hydro-ethanolic extract of E. neriifolia and EN against DENA-induced renal carcinogenicity.

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