PALM OIL TOCOTRIENOLS AS ANTIOXIDANTS AND CHEMOPREVENTIVE AGENTS

FU-LI YU*; ABDUL GAPOR MOHAMAD TOP**; WANDA BENDER* and KATARZYNA BERBEKA*

ABSTRACT
Breast cancer leads all cancer incidence among American women, accounting for 32% of the 2005 estimated new cases in the United States. It is the second leading cause of cancer deaths, estimated at 40 000 yr⁻¹. Estrogens, natural or synthetic, used widely in a variety of clinical conditions, from estrogen replacement therapy to cancer treatment, are themselves carcinogenic, causing uterine and breast cancers. However, the mechanism of their carcinogenic action is still not well understood. We found that both 17β-estradiol (E₂) and estrone (E₁) could be activated by the versatile epoxide-forming oxidant dimethyldioxirane (DMDO) to inhibit nuclear RNA synthesis and to bind DNA forming estrogen-DNA adducts both in vitro and in vivo. Since DNA adducts can cause mutation, and mutation is the molecular basis for the initiation of carcinogenesis, our findings strongly suggest the possibility that both E₂ and E₁ are the initiators for uterine and breast carcinogenesis. Based on this new insight, a method to screen chemopreventive agents against breast cancer, at the initiation, was developed. This screening test determines whether a chemical is able to prevent the formation of E₂ or E₁ epoxide as measured by both the loss of the ability of E₂ or E₁ to inhibit nuclear DNA-dependent RNA synthesis and the ability of [³H]E₂ or E₁ to bind DNA. This article summarizes the results of our recent studies on the preventive effects of Red Palm Oil, tocopherols and tocotrienols on the epoxidation of several carcinogens including E₂, E₁ and aflatoxin B₁ (AFB₁). Our data suggest that tocotrienols are more potent chemopreventive agents than tocopherols against the epoxide formation of E₂, E₁ and AFB₁. As a dietary supplement, tocopherols and especially tocotrienols may have the potential to prevent breast and liver cancers.

INTRODUCTION
Breast cancer leads all cancer incidences among American women, accounting for 32% of the 2005 estimated new cases in the United States (Jemal et al., 2005). It is the second leading cause of cancer deaths, estimated at 40 000 yr⁻¹ (Jemal et al., 2005). Epidemiological studies have shown that 70%-80% of all cancer incidence is due to environmental factors and personal lifestyle (Doll et al., 1981), and is therefore, in theory, preventable. However, in order to prevent a disease, it is necessary to understand the cause of the disease. Estrogens, natural or synthetic, used widely in a variety of clinical conditions, from estrogen replacement therapy to cancer treatment, are themselves carcinogenic, causing uterine and breast cancers (Mgonigle et al., 1994; Grady et al., 1995; Toniolo et al., 1995; Colditz et al., 1998; Schairer et al., 1998; Colditz et al., 1998; Schairer et al., 1998; Rossouw et al., 2002). However, the mechanism of their carcinogenic action is still not well understood. Because estrogens are required for the growth and development of target cells, it has long been believed that estrogens are promoters for carcinogenesis (Feigelson et al., 1996). However, this promotional hypothesis is not able to explain how the cancer cells are developed initially. Chemical carcinogenesis is a multistage process.
including initiation, promotion and progression (Miller et al., 1981; Weisburger, 1980; Farber, 1984; Hemminki, 1993; Dipple, 1995). Initiation is the first critical and irreversible step in carcinogenesis, it requires the covalent binding of a carcinogen to DNA forming DNA adducts (Miller et al., 1981; Weisburger, 1980; Farber, 1984; Hemminki, 1993; Dipple, 1995). DNA adducts can cause mutation and mutation is the molecular basis of carcinogenesis (Miller et al., 1981; Weisburger, 1980; Farber, 1984; Hemminki, 1993; Dipple, 1995).

Several years ago, we found that estrone (E1) and 17β-estradiol (E2) similar to the well established initiating chemical carcinogens, e.g. benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene, aflatoxins, etc. (Miller et al., 1981; Weisburger, 1980; Farber, 1984; Hemminki, 1993; Dipple, 1995; Yu et al., 1994; 1996; Raney et al., 1992; Iyer et al., 1992), could be activated by epoxidation and to bind DNA forming estrogen-DNA adducts both in vitro (Yu et al., 1996; 1998a,b; 1999a) and in vivo (Yu et al., 1998c; 1999b; 2001), and to inhibit nuclear DNA-dependent RNA synthesis (Yu et al., 1996; 1998a, b). These findings strongly suggest that both E1 and E2 are potential initiators for uterine and breast cancers. Based on this new insight, a method to screen chemopreventive agents against breast cancer, at the initiation, was developed (Yu, 2002). This screening test determines whether a chemical is able to prevent the formation of E1 or E2, epoxide as measured by both the loss of the ability of E1 or E2 to inhibit nuclear DNA-dependent RNA synthesis and the ability of [3H]-labelled E1 or E2 to bind to DNA (Yu, 2002). Using this screening protocol, we have found that the breast cancer preventive effect of tamoxifen (TAM) is through a competitive epoxidation mechanism that prevents the formation of E1 and E2 epoxides and consequently, the initiation of breast cancer (Yu et al., 2003). We provided evidence suggesting that the prevention of chemical carcinogen DNA binding and inhibition of nuclear RNA polymerase activity by organosulfur compounds from garlic, e.g. diallyl disulfide (DADS) and diallyl trisulfide (DATS), as the possible mechanisms for their anti-cancer initiation and proliferation effects (Yu et al., 2003). We studied in detail the chemopreventive potentials of vegetable oils and unsaturated fatty acids against breast cancer carcinogenesis at the initiation (Yu et al., 2004). And we provided a molecular explanation why the tocotrienols are more potent cancer preventive agents than tocopherols against the epoxide formation of not only E2 but also of E1 and AFB1.

**MATERIALS AND METHODS**

**Chemicals**

The 17 β-estradiol (E2) and estrone (E1) were purchased from Sigma Chemical Company (St Louis, MO) while [2,4,6,7-^3^H]E2, (94 Ci mmol^-1^), [2,4,6,7-^3^H]E1, (98 Ci mmol^-1^) and 5′-[α-^32^P]GTP (3000 Ci mmol^-1^) were purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Aflatoxin B1 (AFB1), and [3H (G)]AFB1, (29.2 Ci mmol^-1^) were from Moravek Biochemicals (Brea, CA). Red Palm Oil (RPO), α, γ, and δ-tocopherols, α-γ, and δ-tocotrienols were generous gifts from Malaysian Palm Oil Board.

**Isolation of Rat Liver Nuclei and Phenobarbital Induced Liver Microsomes**

Rat liver nuclei were isolated by the hypertonic sucrose method as described previously (Yu, 1974; 1977). The phenobarbital induced liver microsomes were isolated the same way as described before (Yu, 1983; Yu et al., 1986; 1990).

**Assays for the Preventive Effect of α-Tocopherol, α-Tocotrienol and Red Palm Oil (RPO) on the formation of E2 Epoxide as Reflected by the Loss of E2 Inhibition on Nuclear RNA Synthesis After Dimethyl dioxirane (DMDO) Activation in vitro**

The E2, 1 mg, was mixed with α-tocopherol, α-tocotrienol, or RPO in the amount as indicated (Table 1), and reacted with 1 ml DMDO solution prepared according to Adam et al. (1987) at room temperature for 1 hr. The reaction mixture was then vacuum dried and dissolved in 200 ml dimethylsulfoxide (DMSO). For DNA binding, 50 µl of this reaction mixture in DMSO was added to 0.1 ml rat liver nuclei (150 µg DNA), and incubated on ice for 30 min. Then, 0.5 ml of the RNA assay medium [100 mM Tris-HCl (pH 7.9 at 23°C), 2 mM MnCl2, 28 mM 2-mercaptoethanol, 70 mM (NH4)2SO4 and 0.2 mM each ATP, GTP, UTP and CTP] containing 0.1 pCi [α-^32^P]GTP was added to start RNA synthesis at 37°C for 15 min with shaking. At the end of 15 min incubation, RNA synthesis was terminated by the addition of 3 ml of 10% TCA containing 1% pyrophosphate. The radioactive RNA, after TCA precipitation, was collected onto Whatman GF/C filters, which were washed and counted in 5 ml BioSafe II (Research Products International Corp., Mount Prospect, IL) as previously described (Yu et al., 1996; 1998a, b). The specific activity of RNA polymerase was expressed as picomoles [α-^32^P]GMP incorporated/mg DNA.
Activation of [3H]E2 by DMDO for Rat Liver Nuclear DNA Binding

The procedure for [3H]E2 binding to nuclear DNA after DMDO activation was essentially the same as described previously (Yu et al., 2002; 2004; 2005). Briefly, 1 mg E2 containing 10 µCi [3H]E2 was either activated alone, or in the presence of RPO in the amount as indicated (Figure 1) with 1 ml DMDO solution at room temperature for 1 hr. After activation, the samples were vacuum dried. The samples, dissolved in 200 µl DMSO, were mixed with 400 µl of the isolated rat liver nuclei (600 µg DNA), and incubated on ice for 2 hr for DNA binding. The samples were then digested sequentially with RNase (50 µg, 1 hr), pronase (50 µg, 2 hr), and followed by phenol-chloroform extraction of the DNA (Yu, et al., 1995; 2002; 2004; 2005). The DNA in the final aqueous phase was precipitated with 1/10 volume 30% sodium acetate (pH 5.0) and four volumes of 95% ethanol at -20ºC overnight. The DNA pellet was repeatedly washed with 95% alcohol containing 3% sodium acetate to get rid of the free [3H] labelled E2. Finally the DNA from each group was re-dissolved in 0.5 ml H2O for optical density measurement and radioactivity counting.

Activation of [3H]E2 by Liver Microsomes for Calf Thymus DNA Binding

The procedure for the binding of [3H]AFB1 to DNA after activation by liver microsomes as described previously (Yu, et al., 1983; 1986; 1990; 1994; 1996) was adopted for the present study (Figure 2). The 1 mg E2 containing 10 µCi [3H]E2 was mixed either with 100 µl DMSO alone (the control group), or with 100 µl DMSO containing the amount of RPO as indicated (Figure 2). The samples were incubated together with 20 µl microsomes (0.5 mg protein) in buffer A (0.05 M Tri-HCl, pH 7.5 containing 0.25 M sucrose), 20 µl binding buffer (0.9 M Tris-HCl, pH 7.5,0.06 M MgCl2, 16 mM NADP, 100 mM glucose-6-phosphate) containing 0.2 units glucose-6-phosphate dehydrogenase, and 600 µg calf thymus DNA in 100 µl H2O for 1 hr at 37 ºC. The samples were then digested sequentially with RNase A (50 µg, 1 hr), pronase (50 µg, 2 hr), and the DNA was purified and counted as described in Figure 1.

Activation of [3H]AFB1 by DMDO for Calf Thymus DNA Binding

The procedure for [3H]AFB1 binding to calf thymus DNA after DMDO activation was essentially the same as described previously for [3H]E2 binding (Yu et al., 1998a, b; 2003). Briefly, AFB1, 40 µg, containing 5 µCi [3H] labelled AFB1 was incubated either alone or together with 40, 200, or 400 µg α-tocopherol or one of the tocotrienols as indicated (Figure 3) in 0.5 ml DMDO at room temperature for 1 hr. After incubation, the reaction mixtures were vacuum dried. The samples were then dissolved in 160 µl DMSO, together with 160 µl calf thymus DNA (400 µg), 160 µl H2O and incubated on ice for 2 hr.

Figure 1. The preventive effect of Red Palm Oil on the formation of E2 epoxide as reflected by the reduced binding of [3H] labelled E2 to rat liver nuclear DNA after dimethyldioxirane (DMDO) activation in vitro.

Notes: The 1 mg of E2 containing 10 µCi [3H] labelled E2 was mixed with 0, 2, 5 or 10 µl Red Palm Oil as indicated and incubated in 1 ml DMDO at room temperature for 1 hr, and vacuum dried. The samples were dissolved in 200 µl DMSO, mixed with 400 µl of the isolated rat liver nuclei (600 µg DNA), and incubated on ice for 2 hr for DNA binding. The nuclear DNA was then purified after RNase and pronase digestion followed by phenol extraction. The purified DNA from each group was re-dissolved in 0.5 ml H2O for A260 measurement and radioactivity counting. Values given are mean ± SE of 2-3 independent experiments.
Figure 2. The preventive effect of Red Palm Oil on the formation of E2 epoxide as reflected by the reduced binding of \[^{3}H\]labelled E2 to calf thymus DNA after liver microsomes activation in vitro.

Notes: The 1 mg of E2 containing 10 µCi \[^{3}H\]labelled E2 was mixed with 100 µl dimethylsulfoxide (DMSO) alone (the control group), or with 100 µl DMSO containing 0, 2, 5 or 10 µl Red Palm Oil as indicated. The samples were incubated together with 20 µl microsomes (0.5 mg protein) in buffer A (0.05 M Tris-HCl, pH 7.5 containing 0.25 M sucrose), 20 µl binding buffer (0.9 M Tris- HCl, pH 7.5, 0.06 M MgCl\(_2\), 16 mM NADP, 100 mM glucose-6-phosphate) containing 0.2 units glucose-6-phosphate dehydrogenase, and 600 µg calf thymus DNA in 100 µl H\(_2\)O for 1 hr at 37ºC. The samples were then digested sequentially with RNase A (50 µg, 1 hr), pronase (50 µg, 2 hr) and followed by phenol-chloroform extraction of the DNA. The DNA was precipitated, washed, and counted. Values given are mean ± SE of 2-3 independent experiments.

Figure 3. Compare the preventive effect of α-tocopherol, α-, γ- and δ-tocotrienols on the formation of aflatoxin B\(_1\) (AFB\(_1\)) epoxide as reflected by the reduced binding of \[^{3}H\]AFB\(_1\) to calf thymus DNA after dimethyldioxirane (DMDO) activation in vitro.

Notes: The AFB\(_1\), 40 µg, containing 5 µCi \[^{3}H\]AFB\(_1\) was incubated either alone or together with 40, 200 or 400 µg α-tocopherol or one of the tocotrienols as indicated in 0.5 ml DMDO at room temperature for 1 hr. After incubation, the reaction mixtures were vacuum dried. The samples were then dissolved in 160 µl dimethylsulfoxide (DMSO), together with 160 µl calf thymus DNA (400 µg), 160 µl H\(_2\)O and incubated on ice for 2 hr for DNA binding. After binding, 1/10 volume of 30% sodium acetate (pH 5.5) was added to precipitate the DNA. The DNA pellet was washed with 70% alcohol to get rid of free \[^{3}H\]AFB\(_1\). The final DNA precipitate was dissolved in 0.5 ml H\(_2\)O for A\(_{260}\) measurement and radioactivity counting. The \[^{3}H\]AFB\(_1\) + α-tocopherol (●-●-●); \[^{3}H\]AFB\(_1\) + α-tocotrienol (O-O-O); \[^{3}H\]AFB\(_1\) + γ-tocotrienol (×-×-×); \[^{3}H\]AFB\(_1\) + δ-tocotrienol (△-△-△). Values given are means ± SE of 2-3 independent experiments.
for DNA binding. After binding, 1/10 volume of 30% sodium acetate (pH 5.5) was added, and the DNA was precipitated with four volumes of 100% alcohol for 30 min at -20°C. The DNA pellet was washed three times with 2 ml 70% alcohol after each centrifugation. The final DNA precipitate was dissolved in 0.5 ml H₂O for A₂₆₀ measurement and radioactivity counting.

**Activation of [³H]AFB₁ by Liver Microsomes for Calf Thymus DNA Binding**

The procedure for the binding of [³H]AFB₁ to DNA after activation by liver microsomes is essentially the same as described previously (Yu, 1983, Yu, et al., 1986; 1990; 1994; 1996). AFB₁ 40 µg, containing 5 µCi [³H] labelled AFB₁ was mixed with 60 µl DMSO alone, or with 60 µl DMSO containing 40, 200 or 400 µg α-tocopherol or one of the tocotrienols as indicated (Figure 4). Then, 400 µg calf thymus DNA in 170 µl H₂O was added, the samples were incubated together with 10 µl microsomes (0.5 mg protein) in buffer A (0.05 M Tris-HCl, pH 7.5 containing 0.25 M sucrose), and 20 µl binding buffer (0.9 M Tris-HCl, pH 7.5, 0.06 M MgCl₂, 16 mM NADP, 100 mM glucose-6-phosphate) containing 1.0 units glucose-6-phosphate dehydrogenase for 1 hr at 37°C. They were then digested sequentially with a mixture of RNase A (50 µg) and T₁ (5 units) for 1 hr at 37°C, and followed with pronase K (50 µg) for 2 hr at 37°C. The DNA, after phenol-chloroform extraction, was precipitated, washed, and finally dissolved in 0.5 ml H₂O for A₂₆₀ measurement and radioactivity counting.

**Activation of [³H]E₁ by DMDO for Rat Liver Nuclear DNA Binding**

The procedure for [³H]E₁ binding to nuclear DNA after DMDO activation was essentially the same as described previously for [³H]E₂ activation and binding (Yu, et al., 2002; 2004; 2005). Briefly, 1 mg E₁ containing 10 µCi [³H]E₁ was activated either alone, or in the presence of 5 mg of one of the tocopherols

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**Figure 4. Compare the preventive effect of α-tocopherol, α-, γ- and δ-tocotrienols on the formation of aflatoxin B₁ (AFB₁) epoxide as reflected by the reduced binding of [³H]AFB₁ calf thymus DNA after rat liver microsomes activation in vitro.**

Notes: The AFB₁, 40 µg, containing 5 µCi [³H]AFB₁ was mixed with 60 µl dimethylsulfoxide (DMSO) alone, or with 60 µl DMSO containing 40, 200 or 400 µg α-tocopherol or one of the tocotrienols as indicated. Then, 400 µg calf thymus DNA in 170 µl H₂O was added, the samples were incubated together with 10 µl microsomes (0.5 mg protein) in buffer A (0.05 M Tris-HCl, pH 7.5 containing 0.25 M sucrose), and 20 µl binding buffer (0.9 M Tris-HCl, pH 7.5, 0.06 M MgCl₂, 16 mM NADP, 100 mM glucose-6-phosphate) containing 1.0 units glucose-6-phosphate dehydrogenase for 1 hr at 37°C. They were then digested sequentially with a mixture of RNase A (50 µg) and T₁ (5 units) for 1 hr at 37°C, and followed with pronase K (50 µg) for 2 hr at 37°C. The DNA, after phenol-chloroform extraction, was precipitated, washed, and finally dissolved in 0.5 ml H₂O for measurement and radioactivity counting. The [³H]AFB₁ + α-tocopherol (● - - ●); [³H]AFB₁ + α-tocotrienol (O - - O); [³H]AFB₁ + γ-tocotrienol (× - - ×); [³H]AFB₁ + δ-tocotrienol (△ - - △). Values given are means ± SE of 2-3 independent experiments.
or tocotrienols as indicated (Figure 5) with 1 ml DMDO at room temperature for 1 hr, and vacuum dried. The samples, dissolved in 200 µl DMSO, were mixed with 400 µl of the isolated rat liver nuclei (600 µg DNA), and incubated on ice for 2 hr for DNA binding. After binding, the samples were then digested sequentially with a mixture of RNase (100 µg), and T1 (5 units) for 1 hr, and pronase (50 µg) for 2 hr followed by phenol-chloroform extraction of the DNA (Yu et al., 1995; 2002; 2004; 2005). The DNA extract in the final aqueous phase was precipitated with 1/10 volume 30% sodium acetate (pH 5.0) and four volumes of 95% ethanol at -20°C overnight. The DNA pellet was repeatedly washed with 95% alcohol containing 3% sodium acetate to get rid of the free [3H] labelled E1. Finally, the DNA from each group was re-dissolved in 0.5 ml H2O for optical density measurement and radioactivity counting. Values given are mean ± SE of 2-3 independent experiments.

**Table 1** shows that the rate of RNA synthesis for 0.1 ml rat liver nuclei (150 µg DNA) in the presence of 1 mg E2 under the assay conditions used was 634 pmol [32 P]GMP incorporated/mg DNA (100%) after 15 min incubation in vitro. However, the rate of RNA synthesis was reduced to only 209 pmol [32 P]GMP incorporated/mg DNA (33%) after the nuclei was treated with 1 mg E2 activated by DMDO under identical assay conditions. This result is in good agreement with our earlier reports (Yu et al., 1996, 1998a, b; 2002; 2003; 2004; 2005) suggesting that E2 after converting to E2 epoxide by DMDO is able to bind DNA forming DNA adducts impairing the DNA template resulting in the inhibition of DNA-dependent nuclear RNA synthesis. Furthermore, as shown also in Table 1, when nuclei were treated with 1 mg E2 activated by DMDO under identical assay conditions. Values given are mean ± SE of 2-3 independent experiments.
demonstrated by direct [3H]E2 binding to DNA (100%) pmol mg⁻¹ DNA. The binding values were able to bind rat nuclear DNA at a level of 18 930 pmol mg⁻¹. However, the values of the binding were reduced to 18 173 (96%), 10 412 (55%), and 5490 (34%) when 1 mg of E2 containing 10 µCi [3H]E2 was activated by DMDO. As shown in Figure 2, when 1 mg E2 was mixed with 10 µCi [3H]E2, it was able to bind rat nuclear DNA at a level of 18 930 (100%) pmol mg⁻¹ DNA. The binding values were reduced to 18 173 (96%), 10 412 (55%), and 5490 (34%) pmol mg⁻¹ DNA when 2, 5 and 10 µl RPO was mixed with E2 at the very beginning for the activation.

Aflatoxin B1 (AFB₁), a naturally occurring mycotoxin produced by Aspergillus flavus and Aspergillus parasiticus, is a potent rat liver carcinogen (Busby Jr., et al., 1984). Similar to E2, it requires the activation either by microsomal P450 enzymes (Lin et al., 1977; Essigmann et al., 1977; Yu, 1983; Yu et al., 1986) or by the versatile epoxide-forming oxidant DMDO (Baertschi et al., 1988; Yu et al., 1994; 1996) to the ultimate carcinogen AFB₁, exo-8,9-epoxide (AFB₁ epoxide) before it is able to bind DNA forming DNA adducts (Lin, et al., 1977; Essigmann et al., 1977; Baertschi et al., 1988; Yu et al., 1990). This is believed to be the molecular mechanism for AFB₁, epoxide to initiation of liver cancer carcinogenesis. Based on this fact, the method developed for E2 was adopted for AFB₁ to screen chemopreventive agents against liver cancer carcinogenesis at the initiation. This screening test determines whether a chemical is able to prevent the formation of AFB₁, epoxide as measured by the loss of the ability of [3H]AFB₁ to bind DNA.

Table 1 summarizes the preventive effect of α-tocopherol, α-, γ- and δ-tocotrienols on the formation of AFB₁ epoxide activated by DMDO. As the data indicate that when AFB₁, 40 µg, containing 5 µCi [3H]AFB₁ was activated alone by DMDO, the binding of [3H]AFB₁, to calf thymus DNA was 11 123 pmol mg⁻¹ DNA (100%). However, when the activation was carried out in the presence of 40, 200 and 400 µg α-tocopherol, the values of the binding of [3H]AFB₁ to calf thymus DNA were 9455 (85%), 8231 (74%), and 10 901 (98%), respectively. When the activation was carried out in the presence of 40, 200 and 400 µg α-tocotrienol, the values of the binding of [3H]AFB₁ to calf thymus DNA were 10 901 (98%), 8231 (74%), and 8455 (76%), respectively. When the activation was carried out in the presence of 40, 200 and 400 µg γ-tocotrienol, the binding values were 10 901 (98%), 6340 (57%), and 5117 (46%), respectively. And in the presence of 40, 200 and 400 µg δ-tocotrienol, the binding values were 9232 (83%), 5228 (47%), and 4004 (36%), respectively. When the activation was carried out in the presence of 40, 200 or 400 µg α-tocopherol, the values of the binding of [3H]AFB₁, to calf thymus DNA were

### Table 1. The Preventive Effect of α-Tocopherol, α-, γ- and δ-Tocotrienols on the Formation of E₂, E₂-epoxide as Reflected by the Loss of E₂ Inhibition on Nuclear RNA Synthesis after Dimethylsulfoxide (DMDO) Activation

<table>
<thead>
<tr>
<th>Group</th>
<th>Nuclear RNA synthesis* (pmol [32P] GMP incorporated/mgDNA)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>E₂</td>
<td>634 ± 6</td>
<td>100</td>
</tr>
<tr>
<td>E₂ + DMDO</td>
<td>209 ± 6</td>
<td>33</td>
</tr>
<tr>
<td>E₂ + α-tocopherol (5 mg) + DMDO</td>
<td>330 ± 25</td>
<td>52</td>
</tr>
<tr>
<td>E₂ + α-tocotrienol (5 mg) + DMDO</td>
<td>646 ± 13</td>
<td>102</td>
</tr>
<tr>
<td>E₂ + Red Palm Oil (10 µl) + DMDO</td>
<td>468 ± 2</td>
<td>74</td>
</tr>
</tbody>
</table>

Notes: *E₂, 1 mg, was mixed with α-tocopherol, α-tocotrienol, or Red Palm Oil in the amount as indicated, and reacted with 1 ml DMDO at room temperature for 1 hr. The reaction mixture was then vacuum dried and dissolved in 200 µl dimethylsulfoxide (DMSO). Then, 50 µl of this reaction mixture in DMSO was added to 0.1 ml rat liver nuclei (150 µg DNA), and incubated on ice for 30 min. RNA synthesis was assayed in 0.5 ml assay medium containing 0.1 µCi [α–32P]GTP at 37°C for 15 min. The radioactive RNA, after TCA precipitation, was collected onto Whatman GF/C filters, which was washed and counted. Values given are mean ± SE of 3-4 independent experiments.

The evidence that RPO was able to prevent the formation around 50%. RPO is also clearly capable to prevent the activation of E₂ to epoxide by DMDO, but the underlying mechanism is more complicated because it contains several powerful antioxidants such as vitamin A precursor carotenoids, tocopherols, tocotrienols as well as the monounsaturated oleic and polyunsaturated linoleic acids, etc. (Yu et al., 2004).

The results in Figure 1, when 1 mg E₂, containing 10 µCi [3H]E₂, was activated by DMDO, it was able to bind rat nuclear DNA at a level of 18 930 (100%) pmol mg⁻¹ DNA. The binding values were reduced to 18 173 (96%), 10 412 (55%), and 5490 (34%) pmol mg⁻¹ DNA when 2, 5 and 10 µl RPO respectively, was mixed with the 1 mg of [3H]E₂ for epoxidation by DMDO.

The preventive effect of RPO on the formation of E₂, epoxide was further verified using rat liver microsomes as the activation system (Yu et al., 1994; 1996; 1983; 1986; 1990). The results in Figure 2 show that when 1 mg of E₂ containing 10 µCi [3H]E₂, was activated by rat liver microsomes in the presence of 600 µg calf thymus DNA for 1 hr at 37°C, the binding of the [3H]E₂ to DNA was 3912 (100%) pmol/mg DNA. However, the values of the binding were reduced to 2775 (71%), 2228 (57%) and 1682 (43%) pmol mg⁻¹ DNA respectively, when 2, 5 and 10 µl RPO were mixed with E₂ at the very beginning for the activation.
RPO is one of the richest natural sources for carotenoids and vitamin E (Cottrell, 1991; Sambanthanmurthi et al., 2000; Edem, 2002; Sundram et al., 2003). Since both vitamins A and E are known to play an important role in the prevention of many health-related diseases (Sambanthanmurthi et al., 2000; Edem, 2002; Sundram et al., 2003; Radhika et al.; Benade, 2003; Kritchevsky et al., 2002; Stampfer, 1999; Rimm et al., 1993; Marchioli et al., 1999; Pruthi et al., 2001; Yusoff, 2002; Yusuf et al., 2000), including cancer (Gaziano et al., 2004; Gysin et al., 2002; Goh et al., 2002; Rahmat et al., 1993; Ngah et al., 1991), it should be obvious for this basic reason that the use of RPO has significant advantage over other vegetable oils. Our results presented in Table 1 and Figures 1 and 2 have clearly shown that RPO has a strong preventive effect on the formation of E1 epoxide as reflected by the loss of the ability of E1 to bind DNA. Since RPO contains a mixture of the powerful antioxidants including carotenoids, tocopherols and tocotrienols, these may be the major contributors for the observed preventive effect. Another potential contributing factor should be considered is the fact that RPO contains both mono- and polyunsaturated fatty acids. From our earlier studies we have demonstrated that these mono- and polyunsaturated fatty acids are effective competitive epoxidation substrates and are able to prevent E2 epoxidation in vitro (Yu et al., 2004).

AFB1 is a potent liver carcinogen (Busby Jr et al., 1984). Similar to the female hormones E and E2, it requires the activation to AFB1 epoxide before it is able to bind DNA (Lin et al., 1977; Essigmann et al., 1977; Yu, 1983; Yu et al., 1986; Baertschi et al., 1998; 1994; 1996). It is important, for this reason, to find out whether α-tocopherol and tocotrienols are also able to prevent the AFB1 epoxidation and therefore the potential to prevent AFB1-induced liver cancer at the initiation. Results presented in Figures 3 and 4 indicate that α-tocopherol is not effective in the prevention of AFB1 epoxide formation under the experimental conditions used. Tocotrienols, on the other hand, are more effective in the prevention using either DMDO (Figure 3), or rat liver microsomes activation system (Figure 4).

We have reported previously that E2, like E1, could be activated by DMDO to epoxide and to bind DNA (Yu et al., 1988a; b; 2002). It is known that E2 is the major female hormone for post-menopausal women, and since breast cancer incidence increases sharply after menopause (Jemal et al., 2005), it is an interesting but unsettled question whether E2 is causally related to this phenomenon. That aside, it is basically important to find out whether tocopherols and tocotrienols are able to prevent E2 epoxide formation. The results as shown in Figure 5 clearly indicate that both tocopherols and tocotrienols are very effective in the prevention of E2,
epoxide formation, although by comparison tocotrienols are still more effective chemopreventive agents than tocopherols.

The underlying mechanism in the prevention of epoxidation of a chemical carcinogen by chemopreventive agents is believed through a competitive epoxidation inhibition mechanism (Yu, 2002; Yu et al., 2002). We further believe that there are differences in epoxidation potentials among various chemical carcinogens. It is for this reason that tocopherols and tocotrienols are able to effectively prevent the formation of E₂ and E₁ epoxides, and are less effective against the formation of AFB₁ epoxide.

Prevention is the best way to win the war on cancer. In this article, data are presented to show the preventive effect of tocopherols and tocotrienols on the formation of E₂, E₁ and AFB₁ epoxides. As a dietary supplement, tocopherols and especially tocotrienols may have the potential to prevent breast and liver cancers.

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