Inhibition of rat mammary microsomal oxidation of ethanol to acetaldehyde by plant polyphenols

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Abstract
We previously reported that the microsomal fraction from rat mammary tissue is able to oxidize ethanol to acetaldehyde, a mutagenic-carcinogenic metabolite, depending on the presence of NADPH and oxygen but not inhibited by carbon monoxide or other cytochrome P450 inhibitors. The process was strongly inhibited by diphenyleneiodonium, a known inhibitor of NADPH oxidase, and by nordihydroguaiaretic acid, an inhibitor of lipoxygenases. This led us to suggest that both enzymes could be involved. With the purpose of identifying natural compounds present in food with the ability to decrease the production of acetaldehyde in mammary tissue, in the present studies, several plant polyphenols having inhibitory effects on lipoxygenases and of antioxidant nature were tested as potential inhibitors of the rat mammary tissue microsomal pathway of ethanol oxidation. We included in the present screening study 32 polyphenols having ready availability and that were also tested against the rat mammary tissue cytosolic metabolism of ethanol to acetaldehyde. Several polyphenols were also able to inhibit the microsomal ethanol oxidation at concentrations as low as 10–50 μM. The results of these screening experiments suggest the potential of several plant polyphenols to prevent in vivo production and accumulation of acetaldehyde in mammary tissue.

Keywords
ethanol, alcohol, acetaldehyde, breast, mammary, plant polyphenols

Introduction
There is at present significant evidence from epidemiological studies to support a positive association between alcohol drinking and an increase of risk for breast cancer.¹–⁴ In fact, recent epidemiological studies in a total of 1,280,296 middle-aged women in the United Kingdom reported that even drinking women consuming an average of only 10 grams alcohol (one drink) per day evidenced 12% increased risk of breast cancer.⁴ The mechanism mediating this association still remains to be fully understood. Several studies support the hypothesis that ethanol use may increase breast cancer risk at least in part, through an effect in estrogen levels in women, essentially by stimulating mitotic division of already initiated cells.⁵–¹² Further, other factors considered to play a promotional role in the case of ethanol-induced cancer in target organs other than breast (e.g. oxidative stress in the case of liver)³,¹³ could also be involved in the case of mammary tissue. In effect, repetitive administration for 28 days also evidenced the ability of ethanol to promote oxidative stress in mammary tissue.¹⁴

Concerning the nature of the mutational event responsible for the initiation step, recent studies¹¹,¹⁵,¹⁶ suggest that acetaldehyde produced elsewhere (e.g. in liver) and arriving at mammary tissue via blood or produced by metabolic transformation in situ¹⁴,¹⁷–¹⁹ could
be a key putative initiating agent of the ethanol-promoted breast cancer. Recent studies from our laboratory strongly suggested that acetaldehyde tends to accumulate in mammary tissue during long periods of time after single doses of ethanol given orally. The intensity of the accumulative process and the period of time during which it can be observed depend on the dose of ethanol administered, while levels of acetaldehyde in plasma were similar at all the different ethanol doses given. These results suggest that ability of mammary tissue to oxidize ethanol in situ and its lack to detoxify further the acetaldehyde would be a major player in the acetaldehyde accumulation process.

In previous studies by Guerri and Sanchis, the authors did not detect any alcohol dehydrogenase (ADh) activity in whole homogenates of rat mammary tissue. In our hands, only traces of ADh activity was evidenced in the cytosolic fraction and they were far smaller than the one present in the liver. Further, Triano et al. reported that human mammary tissue contains class I ADh having a limited potential to metabolize ethanol to acetaldehyde.

There is, however, another relevant pathway in cytosolic fraction contributing to acetaldehyde production from ethanol present in rat mammary tissue that is mediated by xanthine oxidoreductase (XOR). This metabolic pathway is able not only to metabolize ethanol to acetaldehyde but also to generate hydroxyl radicals and it is also induced by repetitive alcohol drinking.

In previous studies from our laboratory, we reported that this XOR-mediated cytosolic pathway can be effectively inhibited by several plant polyphenols and by folic acid.

Further work also showed that the microsomal fraction also oxidizes ethanol to acetaldehyde. In effect, there was a pathway depending on the presence of NADPH and oxygen but it was not inhibited by carbon monoxide or SKF 525A (N,N-diethylaminoethyl 2,2-diphenylvalerate). That excluded participation of cytochrome P450 but was potently inhibited by diphenyleneiodonium (DPI), a known inhibitor of NADPH oxidase, and by nordihydroguaiaretic acid (NDHGA), a general inhibitor of lipoxygenases. This led us to suggest that both enzymes could be involved in the microsomal metabolism of ethanol to acetaldehyde. In the present studies, several plant polyphenols having inhibitory effects on lipoxygenases and of antioxidant nature were tested as potential inhibitors of the above described rat mammary tissue microsomal pathway of metabolic transformation to acetaldehyde.

The experiments would also include all the polyphenols previously shown to inhibit the XOR-cytosolic pathway of ethanol metabolism present in the rat mammary tissue.

The reason for including them is that if antioxidant polyphenols previously showed to inhibit the XOR-mediated oxidation of ethanol to acetaldehyde in the cytosolic fraction also inhibited the microsomal pathway, reasonable future perspectives for preventive effects in vivo experiments could be expected.

Materials and methods

Chemicals

Absolute ethanol (analytical grade) was from Sintorgan (Buenos Aires, Argentina). Acetaldehyde was from Fluka (Buchs, Switzerland). The compounds tested for their effects on the metabolism of ethanol were of the best quality available: nordihydroguaiaretic acid; quercetin dihydrate, (+)-naringenin, naringin, (+)-catechin, (-)-catechin, (+)-epicatechin, (-)-epigallocatechin gallate (EGCG), ellagic acid, resveratrol, caffeic acid and its phenethyl ester, curcumin, propolis extract (from propolis collected in the state of Pennsylvania), hesperetin, pelargonidin chloride, ferulic acid, kaempferol, myricetin, apigenin, genistein, and morin were from Sigma Co. (St. Louis, Missouri, USA). Baicalein was from Aldrich Co. (Milwaukee, Wisconsin, USA). Daidzein, fisetin, formononetin, chlorogenic acid, and enterodiol were from Fluka (Buchs, Switzerland).

Animals and treatments

Non-inbred female Sprague Dawley rats (18 weeks, 220–260 g) were used. The rats were postlactation young mothers (2 weeks after weaning of their pups). The procedures used for breeding, housing, and handling animals were those established by the Food, Drug and Medical Technology National Administration (ANMAT; Buenos Aires, Argentina). The starting breeding colony was from Charles River (Wilmington, Massachusetts, USA). They were fasted for 12–14 hours before sacrifice, but water was available ad libitum. Animals were killed by decapitation, and their mammary tissue was rapidly excised and processed.

Isolation of the microsomal fraction from mammary tissue

Mammary tissue was homogenized via
Teflon-glass Potter-Elvehjem homogenizer with 4 volumes of STKM buffer (0.25 M sucrose in TKM buffer: 50 mM Tris-HCl, 5 mM MgCl₂, and 2.5 mM KCl, pH 7.5). Procedure was performed at 4°C. The homogenates were centrifuged at 9000 g for 20 min. The resulting supernatants were centrifuged at 105,000 g for 1 hour and the microsomal pellets were recovered. These preparations were essentially free from cross-contamination with other cellular fractions.¹⁴,¹⁹

**Ethanol metabolism to acetaldehyde in the microsomal fraction** Preparations containing microsomes (0.26 ± 0.05 mg protein/mL), NADPH generating system (0.45 mM NADP⁺, 4 mM d,l-isocitric acid trisodium salt and 0.25 units of isocitric dehydrogenase), and 0.14 M ethanol in 50 mM KH₂PO₄, pH 7.4 (3 mL final volume), were incubated for 1 hour at 37°C under air. Plant polyphenols were solubilized in the alcohol or in water. Three samples per group were run, each consisting of microsomes from a separate lot of pooled mammary tissue (five animals each). Incubations were performed in aluminum-sealed neoprene-septum-stoppered glass vials. The reaction was terminated by plunging in ice. After adding 1 mL of saturated NaCl solution, samples were kept at 37°C for 10 min and an aliquot (100 μL) of the headspace was analyzed by gas chromatography with flame ionization detector (GC-FID). Chromatographic conditions were column, GS-Q, 25 m × 0.53 mm i.d. (J&W Scientific, California, USA); temperature 110°C isothermal, injection port temperature: 150°C, FID: 200°C.¹⁴,¹⁹

**Statistics** The significance of the difference between mean values was assessed by unpaired t test (Student’s t test).²⁶ Calculations were performed using GraphPad Software. Differences were considered significant when p < 0.05.

**Results**

**Effect of plant polyphenols on the bioactivation of ethanol to acetaldehyde by rat mammary tissue microsomal fraction**

Acetaldehyde levels in incubation mixtures containing the microsomal fraction of rat mammary tissue are summarized in Table 1. The NADPH-mediated ethanol activation pathway was strongly inhibited by fisetin (94%), apigenin (86%) and naringenin (80%), and by the well-known inhibitor of lipoxygenases NDHGA (60%) at concentrations as low as 10 to 50 micromolar. At those concentrations, other polyphenols were also able to significantly decrease the production of acetaldehyde: kaempferol (49%), luteolin (47%), genistein (45%), hesperetin (46%), caffeic acid (41%), pelargonidin (43%), and ferulic acid (36%). At a lower extent, inhibitory effects were also observed at the same concentrations with myricetin (22%), daidzein (26%), (+)-catechin (22%), (-)-catechin (34%), (+)-epicatechin (32%), naringin (20%), caffeic acid phenetyl ester (28%), resveratrol (22%), and ellagic acid (20%). Other polyphenols showed little or no inhibitory ability, for example, morin, quercetin, formononetin, baicalein, chlorogenic acid, curcumin, epigallocatechin gallate, or enterodiol.

**Discussion**

Recent studies from our laboratory strongly suggested the significance of alcohol metabolism to acetaldehyde in situ and the marked accumulation of the latter in mammary tissue.¹⁴,¹⁸-²⁰ The accumulation of acetaldehyde in mammary tissue would be of particular significance considering that it is a mutagenic and carcinogenic chemical able to react not only with DNA but also with other components relevant to cellular function such as proteins, lipids, glutathione, and other targets.²⁷-³⁰

Recent comprehensive analysis about the mechanisms of the carcinogenic effects of alcohol drinking on different organs from the aerodigestive tract, liver, and others paid particular emphasis about the role of acetaldehyde formation or presence in those tissues as a major player in the process ending in cancer.³

In our previous studies, we evidenced the presence in the rat mammary tissue of at least two metabolic pathways of generation of acetaldehyde. One is present in the cytosolic fraction and is mediated by XOR.¹⁸ The other is present in the microsomal fraction, requiring NADPH and oxygen not being P450 dependent or mediated by catalase or cyclooxygenase but strongly inhibited by DPI and NDHGA. Because of the potent inhibitory effect of NDHGA, the participation of a lipoxygenase was hypothesized in those studies.¹⁹ In effect, NDHGA is a known inhibitor of lipoxygenases.²⁴,²⁵ On the other hand, we hypothesized that the inhibitory effect of DPI could be interpreted as suggesting the additional participation of an NADPH oxidase enzyme as a supplier of hydrogen peroxide. Under this view, the role of NADPH oxidase would be the generation of the necessary cosubstrate required by
Table 1. Effect of plant polyphenols on the biotransformation of ethanol to acetaldehyde in the microsomal fraction of rat mammary tissue

<table>
<thead>
<tr>
<th>Compound</th>
<th>Acetaldehyde (nmol) / protein (mg)</th>
<th>Acetaldehyde (nmol) / protein (mg)</th>
<th>% Inhibition&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ NADPH</td>
<td>- NADPH</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>2.97 ± 0.59</td>
<td>1.30 ± 0.18</td>
<td>0</td>
</tr>
<tr>
<td>Flavonols</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μM fisetin</td>
<td>0.68 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.58 ± 0.05</td>
<td>94</td>
</tr>
<tr>
<td>10 μM kaempferol</td>
<td>1.57 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.72 ± 0.03</td>
<td>49</td>
</tr>
<tr>
<td>10 μM myricetin</td>
<td>2.78 ± 0.12&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.48 ± 0.12</td>
<td>22</td>
</tr>
<tr>
<td>50 μM morin</td>
<td>2.12 ± 0.08&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.54 ± 0.08</td>
<td>5</td>
</tr>
<tr>
<td>10 μM quercetin</td>
<td>2.75 ± 0.06&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.35 ± 0.03</td>
<td>16</td>
</tr>
<tr>
<td>Flavones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μM apigenin</td>
<td>0.81 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.57 ± 0.03</td>
<td>86</td>
</tr>
<tr>
<td>10 μM baicalein</td>
<td>3.46 ± 0.12</td>
<td>1.55 ± 0.03</td>
<td>0</td>
</tr>
<tr>
<td>10 μM luteolin</td>
<td>1.36 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.47 ± 0.02</td>
<td>47</td>
</tr>
<tr>
<td>Isoflavones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μM daidzein</td>
<td>2.59 ± 0.11&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.36 ± 0.06</td>
<td>26</td>
</tr>
<tr>
<td>10 μM formononetin</td>
<td>2.92 ± 0.16&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.15 ± 0.1</td>
<td>0</td>
</tr>
<tr>
<td>10 μM genistein</td>
<td>2.47 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.55 ± 0.08</td>
<td>45</td>
</tr>
<tr>
<td>Flavanols</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 μM (+)-catechin</td>
<td>2.72 ± 0.06&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.42 ± 0.01</td>
<td>22</td>
</tr>
<tr>
<td>10 μM (-)-catechin</td>
<td>2.45 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.35 ± 0.1</td>
<td>34</td>
</tr>
<tr>
<td>10 μM (+)-epicatechin</td>
<td>2.55 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.42 ± 0.08</td>
<td>32</td>
</tr>
<tr>
<td>10 μM EGCG</td>
<td>2.87 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.40 ± 0.08</td>
<td>12</td>
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<tr>
<td>Flavanones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μM hesperetin</td>
<td>2.28 ± 0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.13 ± 0.23</td>
<td>31</td>
</tr>
<tr>
<td>50 μM hesperetin</td>
<td>2.05 ± 0.02&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.15 ± 0.11</td>
<td>46</td>
</tr>
<tr>
<td>10 μM naringenin</td>
<td>2.76 ± 0.14&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.51 ± 0.07</td>
<td>25</td>
</tr>
<tr>
<td>50 μM naringenin</td>
<td>1.21 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.88 ± 0.03</td>
<td>80</td>
</tr>
<tr>
<td>50 μM naringin</td>
<td>2.65 ± 0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.32 ± 0.01</td>
<td>20</td>
</tr>
<tr>
<td>Phenolic acids and derivatives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μM caffeic acid</td>
<td>2.30 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.31 ± 0.08</td>
<td>41</td>
</tr>
<tr>
<td>10 μM caffeic acid phenethyl ester</td>
<td>2.44 ± 0.08&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.24 ± 0.04</td>
<td>28</td>
</tr>
<tr>
<td>10 μM chlorogenic acid</td>
<td>3.41 ± 0.02</td>
<td>1.35 ± 0.07</td>
<td>0</td>
</tr>
<tr>
<td>10 μM curcumin</td>
<td>3.12 ± 0.13</td>
<td>1.44 ± 0.06</td>
<td>0</td>
</tr>
<tr>
<td>10 μM ferulic acid</td>
<td>2.47 ± 0.05&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.40 ± 0.08</td>
<td>36</td>
</tr>
<tr>
<td>Propolis extract (5 μg/ml)</td>
<td>2.67 ± 0.22&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.15 ± 0.14</td>
<td>9</td>
</tr>
<tr>
<td>Stilbenes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μM resveratrol</td>
<td>2.89 ± 0.05&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.58 ± 0.19</td>
<td>22</td>
</tr>
<tr>
<td>Lignans</td>
<td></td>
<td></td>
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<tr>
<td>10 μM enterodiol</td>
<td>2.96 ± 0.18&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.41 ± 0.05</td>
<td>7</td>
</tr>
<tr>
<td>Anthocyanidins</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>10 μM pelargonidin</td>
<td>2.51 ± 0.06&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.56 ± 0.04</td>
<td>43</td>
</tr>
<tr>
<td>Other polyphenolics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μM ellagic acid</td>
<td>2.6 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.27 ± 0.05</td>
<td>20</td>
</tr>
<tr>
<td>10 μM NDHGA</td>
<td>2.09 ± 0.03&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.42 ± 0.01</td>
<td>60</td>
</tr>
<tr>
<td>100 μM NDHGA</td>
<td>1.31 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.83 ± 0.08</td>
<td>71</td>
</tr>
</tbody>
</table>

<sup>a</sup> Incubation mixtures containing microsomal fraction (0.26 ± 0.05 mg of protein/mL), 0.14 M ethanol, and, when indicated, NADPH generating system, in KH<sub>2</sub>PO<sub>4</sub> buffer, were conducted for 1 hour at 37°C. Acetaldehyde was measured by gas chromatography with flame ionization detector (GC-FID) in the head space of each sample after adding 1 mL NaCl saturated solution. See Materials and Methods section for details. Each result is the mean of three separate lots of pooled mammary tissue samples.

<sup>b</sup> Percentage inhibition was calculated with respect to the "Ethanol" group as the control. Each data of the blank group (–NADPH) was subtracted from the corresponding experimental group (+ NADPH) and then compared to the corresponding difference in "Ethanol" group.

<sup>c</sup> p < 0.0001, compared to air + NADPH.

<sup>d</sup> p < 0.0005, compared to air + NADPH.

<sup>e</sup> p > 0.05, compared to air + NADPH.

<sup>f</sup> p < 0.05, compared to air + NADPH.
lipoxigenase to exert its hydroperoxidase-peroxidase activity against xenobiotics. 31-34

On behalf of this hypothesis is the fact that the specific inhibitory effect on NADPH oxidase of DPI is well established. 23 In the present study, we attempted to provide additional evidence for the participation of a lipoxygenase in the microsomal pathway of ethanol metabolism to acetaldehyde. This attempt was made by testing for the inhibitory effects of polyphenols having the potential for future preventive use in vivo. The reason for that attempt rested in the fact that flavonoids have inhibitory effects on prooxidant enzymes like lipoxigenase and xanthine oxidase. 35

However, it is important in this respect to take into account that plant polyphenols generally have low oral bioavailability. 36,37 Consequently, it is not always feasible to achieve under in vivo conditions those concentrations that were effective under in vitro testing.

That difference might be particularly relevant when the source of the plant polyphenols is the diet. Polyphenols are widely present in fruits, vegetables, and beverages derived from plants (e.g., tea, red wine). Notwithstanding, they have also been administered at pharmacological levels as dietary supplements or herbal remedies. 37

Having these considerations in mind, we considered suitable for these preliminary screening studies to test a significant number of plant polyphenols having inhibitory properties against lipoxigenase and being effective antioxidants on their ability to inhibit the microsomal pathway of ethanol oxidation to acetaldehyde in the rat mammary tissue. 35,38-42 We considered of our particular interest those plant polyphenols that significantly inhibited that metabolism at 10 to 50 µM concentrations, assuming that concentrations of that order of magnitude can reasonably be reached under in vivo experimental conditions. 36,42 Their structures are depicted in Figure 1.

We included in the present screening study 32 plant polyphenols among the more than 8000 compounds having a flavonoid structure that have been identified by 2002. 43 They were selected because of their ready availability and because they were previously tested against the rat mammary tissue cytosolic metabolism of ethanol to acetaldehyde. 22 The test included representative members of chalcones, flavones, flavonols, flavanones, flavanols, anthocyanidins, isoflavones, phenolic acids and their derivatives, stilbenes, and lignanes. Only 6 of the 32 compounds tested failed to inhibit ethanol microsomal oxidation at 10 or even 50 µM concentration (morin, baicalein, formononetin, chlorogenic acid, curcumin, and enterodiol).

All the other polyphenols tested inhibited the metabolism of ethanol to acetaldehyde at 10–50 µM concentration. Some of them were considered in the literature as specific or general inhibitors of lipoxigenases when tested at those concentrations, for example, NDHGA and esculetin. 31 In addition, flavonoids have inhibitory effects not only on lipoxigenases but also on other prooxidant enzymes such as xanthine oxidase, 35,38,44-48 and consequently it is not unexpected that some of here-studied flavonoids inhibited the microsomal pathway of ethanol metabolism to acetaldehyde and the previously reported by our laboratory, the cytosolic XOR-mediated one. 22

That is, the present and past studies on the microsomal pathway of ethanol metabolism to acetaldehyde by mammary tissue further suggest the participation of a lipoxigenase and the additional contribution of NADPH oxidase as a hydrogen peroxide generating enzyme. 19 This hypothesis could explain the inhibitory effect of the microsomal pathway of ethanol metabolism by flavonoids like quer cetin, naringenin, or resveratrol that were reported previously by Pinto and Macias as inhibitors of the hydroperoxidase-dioxygenase activity of soybean lipoxigenase in the presence of H 2O2. 54 That hypothesis visualizes the overall process of microsomal ethanol oxidation to acetaldehyde in rat mammary tissue as a cooperative mechanism between NADPH oxidase and lipoxigenase for ethanol oxidation to acetaldehyde:

\[
\text{NADPHoxidase : NADPH + H}^+ + O_2 \\
\rightarrow \text{NADP}^+ + H_2O_2
\]

\[
\text{Lipoxygenase : H}_2O_2 + CH_3CH_2OH \\
\rightarrow 2H_2O + CH_3CHO
\]

All the polyphenols being potent inhibitors of the mammary tissue microsomal pathway of ethanol metabolism to acetaldehyde were previously evidenced by others as inhibitors of given lipoxigenases. NDHGA and esculetin were repetitively mentioned in literature as general lipoxigenase inhibitors and that further supports the hypothesis that a lipoxigenase is involved in the microsomal pathway of ethanol oxidation. 31 More difficult appears to be at present to establish which is the specific lipoxigenase involved in the process. There were described in literature...
several lipoxygenase members (e.g., the 5-, 8-, 12-, and 15-lipoxygenase). Further, some of the inhibitors exhibited cross reactivity between the different lipoxygenases, when acting at different concentrations and in preparations derived from different origins. To establish the nature of the different lipoxygenases that could be involved, additional experiments are needed and many are in course in our laboratory. Among the plant polyphenols tested here, there are several compounds which in previous work by others showed their ability to inhibit the activation of either necrogenic or carcinogenic chemicals or that they prevented tumor genesis in culture or under in vivo conditions.

Those beneficial effects could be attributed to actions on the activations and detoxications of given procarcinogens or to antiproliferative actions or to proapoptotic effects on cancer cells or to inhibition of tumor angiogenesis. Several plant polyphenols tested in this work and previously by our laboratory were reported by others to have some of those beneficial properties. That included baicalin; caffeic acid; NDHGA; curcumin; chlorogenic acid; ferulic acid; EGCG; epicatechin; catechin; resveratrol, myricetin, apigenin; luteolin; kaempferol; genistein; ellagic acid; quercetin; morin; fisetin, esculetin; silymarin, rutin; daidzein; naringin; and naringenin.

Whether the promising properties of these plant polyphenols tested in the present studies about the inhibition of the ethanol oxidation in rat mammary tissue microsomes and those previously reported for the XOR-mediated cytosolic one leads to preventive actions on the ethanol carcinogenic effects on the breast remains to be established. However, acetaldehyde is known to be a highly mutagenic chemical and was recently considered to be a key putative product involved in ethanol-induced carcinogenic effects, including mammary tissue. Oxidative stress promotion and estrogen-mediated effect were also believed to play a role. The antioxidant properties and the antiestrogenic properties of some of the polyphenols tested (e.g., daidzein and genistein) might offer an additional preventive contribution against alcohol-induced mammary cancer. However it is unknown at present. Notwithstanding, all the above-considered possibilities deserve special attention considering the major incidence of breast cancer in women around the world and the fact that only two etiological factors are known to

Figure 1. Structures of some of the polyphenolic compounds with the most powerful inhibitory ability on the microsomal oxidation of ethanol to acetaldehyde in rat mammary tissue.
be involved in breast cancer in humans: radiation and alcohol consumption. Both are of preventable nature.

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**References**


