CHAPTER 8

Study on the ameliorative potential of gentisic acid on chemically and UVB mediated biochemical alterations in Swiss mice
Primary prevention includes the cessation of contact with the causative factors of a disease. Practically, the feasibility of such an approach is poor. On the other hand, secondary prevention or what is more commonly called as chemoprevention is quite successful and has shown promising results against carcinogenesis. The chemopreventive agents might belong to entirely unrelated class of chemicals like coumarins, azo dyes, sulfur compounds, flavones, phenols, indoles, retinoids, tocochromans, selenium compounds and protease inhibitors (Prochaska et al 1985).

In one of the earlier studies (described in Chapter 5), the chemopreventive effect of Hibiscus rosa-sinensis extract on the marker parameters of tumor promotion and oxidative stress is shown. Oxidative stress, ODC activity and DNA synthesis are invariably induced in case of tumor promotion as is also observed in case of single topical applications of different promoters such as 12-O-tetradecanoyl phorbol-13-acetate, benzoyl peroxide and ultraviolet radiations. These short-term effects of TPA, BPO and UVR are of great significance as they represent a part of the complex process that takes place over a course of time. If these short-term alterations can be blocked then cancer formation can be delayed to a certain extent. Out of the three stages of cancer, initiation is an irreversible stage while progression marks the last stage of cancer events, so the only step, which can be promising in the prevention of cancer, is tumor promotion that is reversible in early stages (DiGiovanni 1992). Tumor promotion stage features only epigenetic changes that include alterations in the biochemistry and histopathology of the tissue. The biochemical parameters associated with tumor promotion include alterations in above said parameters such as the activities of certain enzymes; enhanced synthesis of DNA, RNA and proteins; oxidative insult to the cells (Perchellet and Perchellet 1989).
With the application of the toxicant alone, the level of cellular antioxidant, glutathione is also depleted along with the depletion of its metabolizing enzymes. The increased level of MDA is an indicator of increased peroxidation of lipids. Therefore, MDA and enhanced hydrogen peroxide formation in the TPA, BPO and UVR treated groups act as direct indicators of oxidative damage that might lead to genotoxicity or cytotoxicity in cells (Sun 1990). The extract of Hibiscus rosa sinensis used in one of the studies described earlier showed promising results against two-stage skin carcinogenesis. Keeping this in view, we analyzed the effects of one of its chemical constituents. The extract of the plant possesses various compounds including quercetin, carotene, niacin, riboflavin, malvalic acid, gentisic acid, margaric acid and lauric acid (Ross 1999). Since quercetin, carotene, niacin have been studied extensively our aim was to find out whether or not other chemical constituents also contribute to the modulatory activities of medicinal plants. Mostly, the research work focuses on the effects of the major principle or the active constituent of the plant extract and virtually very few minor constituents draw the attention of the researchers. However, we were particularly interested in one of the minor constituent, gentisic acid of the plant studied earlier. Chemically it is 2, 5-dihydroxy benzoic acid (2, 5 DHBA), which is a phenolic acid. It is basically an adduct product of salicylic acid and hydroxyl radical, therefore, is generated \textit{in vivo} as well. Besides, Radtke et al (1998) have shown that the total sum of hydroxybenzoic acids amounts to 11mg/ day with 75% of salicylic acid intake by plant derived foodstuff including fruits and juices. While, Hinz et al report that it accounts for less than 5% of the ingested salicylic acid (Hinz et al 2000). Chronic arthritis especially in children leads to nutritional impairments, which may start with food allergies and extend to chronic malnutrition. In such cases the biotransformation of salicylic acid to gentisic acid is adversely affected and the levels of gentisic acid is depleted (Lares-Asseff et al 1999).
Gentisic acid is also present as normal constituent of serum and therefore it is important to find answers for questions regarding its role in the promotion of health (Paterson et al. 1998). Most phenolic acids have antioxidant and anticarcinogenic properties. It is noteworthy here that although the salicylate metabolized to gentisic acid is very less, it has been shown to be involved in the antioxidant activities and it is also formed during inflammatory conditions by the polymorphonuclear leucocytes that produce reactive oxygen radicals (Hinz et al. 2000). Other scientists have also shown evidences that it possesses antioxidant, anti-inflammatory and anti-mutagenic properties (Krizkova et al. 2000, Uhrig et al. 2000).

Based on these facts we speculated the role of gentisic acid against TPA and the combination of BPO and UVB induced tumor promotion response and oxidative stress in mice and hence proceeded with the present study. The assessment of antioxidant activity and anti-promotional markers were assessed using the same parameters as described in Chapters 4-7.

TREATMENT PROTOCOL

Short-term studies with gentisic acid against BPO treatment & UVB irradiation induced biochemical changes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment Regiment</th>
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<tr>
<td></td>
<td>Day 1</td>
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<tr>
<td>I</td>
<td>Vehicle</td>
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<td>II</td>
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<td>IV</td>
<td>Vehicle</td>
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<tr>
<td>V</td>
<td>Gentisic acid (D1)</td>
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<tr>
<td>VI</td>
<td>Gentisic acid (D2)</td>
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<tr>
<td>VII</td>
<td>Gentisic acid (D2)</td>
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Vehicle [Acetone: Methanol, 9:1] = 0.2ml/animal, Gentisic acid (D1) = 2.0μg /0.2ml vehicle/animal, Gentisic acid (D2) = 4.0μg /0.2ml vehicle/animal. Doses of BPO and UVB; age and number of the animals; the time period between the gentisic acid pretreatment and BPO + UVB exposure; and the time of sacrifice of all the animals for the estimation of different parameters was similar to that described in Chapter 4.

**Short-term studies with gentisic acid against TPA induced biochemical changes**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
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<tr>
<td>I</td>
<td>Vehicle</td>
<td>Vehicle</td>
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<tr>
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<td>Vehicle</td>
<td>Vehicle</td>
<td>TPA</td>
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<tr>
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<td>IV</td>
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<td>Gentisic acid (D2)</td>
<td>Gentisic acid (D2) + TPA</td>
</tr>
<tr>
<td>V</td>
<td>Gentisic acid (D2)</td>
<td>Gentisic acid (D2)</td>
<td>Gentisic acid (D2)</td>
</tr>
</tbody>
</table>

Vehicle, Gentisic acid (D1) and Gentisic acid (D2) are same as described above. TPA = 20nmol/0.2ml/animal. Each group contains six mice. TPA = 20nmol/0.2ml/animal. Sequence of treatments on the third day and the time of sacrifice of the animals were same as that described in Chapter 4.

**RESULTS**

*Effect of gentisic acid on BPO & UVB mediated oxidative stress and hyperproliferation response*

Figure 1 depicts effect of prophylactic treatment of gentisic acid on BPO treated and UVB mediated depletion in reduced glutathione and glutathione reductase in murine skin. BPO and UVB caused 65 and 71% in reduced glutathione and glutathione reductase respectively.
Prophylaxis with gentisic acid caused 3-11% and 15-20% recovery at low and high doses respectively in case of reduced glutathione and glutathione reductase.

Figure 2 shows effect of prophylaxis of gentisic acid on BPO treated and UVB mediated depletion in cutaneous glutathione-S-transferase and quinone reductase in mice. BPO plus UVB irradiation caused 65 and 70% reduction in glutathione-S-transferase and quinone reductase respectively. Prophylaxis with gentisic acid caused 7-21 and 16-28% at both the doses.

Figure 3 shows effect of pretreatment with gentisic acid on BPO and UVB mediated depletion of catalase, glutathione peroxidase and glucose-6-phosphate dehydrogenase in skin. The depletion observed in case of catalase, glutathione peroxidase and glucose-6-phosphate dehydrogenase was 58, 69 and 68%. Pretreatment with gentisic acid, however, recovered their activities by 4-10 and 14-21% at low and high doses respectively.

Figure 4 shows that treatment with BPO and UVB induced hydrogen peroxide formation (121%) and lipid peroxidation (231%) in murine skin whereas pretreatment with gentisic acid caused 10-14% and 58-71% reduction respectively in the levels of the above said parameters.

Figure 5 depicts the effect of gentisic acid in the attenuation of BPO and UVB induced ODC activity and DNA synthesis. The activity of ODC and the synthesis of DNA increased to 261 and 204% in BPO treated and UVB irradiated group; 231 and 184% in the only BPO treated group; and 150 and 126% in only UVB treated group respectively. While, pretreatment of gentisic acid caused 131 and 75% decline in the elevated ODC activity and 40-68% inhibition in case of DNA synthesis respectively.

Effect of gentisic acid on TPA mediated oxidative stress and hyperproliferation response

Figure 6 shows the moulatory effect of gentisic acid on TPA mediated depletion of cutaneous reduced glutathione content, glutathione-S-transferase and glutathione reductase. Only TPA
application to mice caused 60, 47 and 47% decrease in reduced glutathione content, glutathione-
S-transferase and glutathione reductase respectively. Pretreatment with gentisic acid however,
caused 10-21% recovery at low dose and 18-28% recovery at high dose.

Figure 7 exhibits pretreatment effect of gentisic acid on TPA mediated depletion of catalase,
glutathione peroxidase and glucose-6-phosphate dehydrogenase in skin. The activities of
catalase, GPx and G6PD were significantly depleted on application of TPA to the dorsal shaven
portions of the mice skins to 31, 58 and 48% respectively. Pretreatment with gentisic acid on the
other hand, caused 8-15% recovery at low dose and 22-40% recovery at high dose.

Figure 8 shows that malondialdehyde formation (207%) and H₂O₂ content (177%) were
significantly elevated in TPA treated group. Gentisic acid pretreatment caused 44 & 56%
decrease in MDA formation and caused 27 & 39% recovery in H₂O₂ content in a dose
dependent manner.

Effect of gentisic acid on the induction of ornithine decarboxylase and DNA synthesis in mouse
skin by TPA is shown in Figure 9. There was 5.8-fold increase in ODC activity and 2.4-fold
increase in thymidine incorporation in DNA on treatment of animals with single topical
application of TPA on mice skins. The activity of ODC was restored in case of gentisic acid but
not in a dose dependent manner. On pretreatment with gentisic acid the recovery in [³H]
thymidine incorporation into DNA was 77 & 88%, which was dose dependent.

DISCUSSION

Gentisic acid is one of the components of Hibiscus rosa sinensis extract, plant that has already
been described in Chapter 5. Other aspect that makes it more interesting for study is the fact that
it can be generated in vivo also. In most of the studies, the formation of hydroxyl radical is
quantitated in terms of the increased amounts of 2,3 and 2,5 hydroxybenzoic acid that are
hydroxylative products of salicylic acid (Prasad 1997). It is a second line metabolite of aspirin (Exner et al 2000). It is also used in cosmetics as a skin-whitening agent (Bian et al 2003). Gentisic acid (2,5 hydroxybenzoic acid), on its part, has been shown to possess antioxidant properties in various studies as it scavenges free radicals (Liu and Edwards 2001). It significantly inhibits the formation of PGE2 at concentrations in which it is formed in arthritis (free-radical mediated disease) patients (Hinz et al 2000). It has anti-mutagenic activity (Hinz et al 2000) as well, although no substantial amount of work has been done to check the efficacy of this compound against tumor promotion. In the current study also, we have evaluated the potential of the test agent using two mechanisms of anti-tumor promotion i.e. inhibition of oxidative stress and polyamine biosynthetic parameters.

The results of the present study show that gentisic acid potentially prevents the oxidative damage induced by different promoters. All the three agents used in this study induce oxidative stress. During such a stress, superoxide anion is the first ROS to be generated and if it is not prevented from generation it leads to the formation of highly reactive species, hydroxyl radicals, that attack proteins and lipid membranes causing cell damage or genetic alterations. Lipid peroxidation and hydrogen peroxide are therefore, the markers for assessing the extent of damage in cells (Zhao et al 2000). Gentisic acid helps in the restoration of intracellular antioxidant, reduced glutathione and antioxidant enzymes together with the inhibition of lipid peroxidation and hydrogen peroxide generation in a dose dependent manner. Gentisic acid like other modulators successfully prevented oxidative stress induced not only by the combination of BPO and UVB but also by the individual strong tumor promoter, TPA. It restored the cellular glutathione content and the activities of antioxidant enzymes like catalase and glutathione peroxidase. Additionally, it also inhibited
the epidermal peroxidation of lipids and the formation of hydrogen peroxide that would otherwise have lead to the formation of reactive species. Several naturally occurring chemopreventive agents also inhibit the formation of $H_2O_2$ and oxidized DNA bases (Huang et al 1997). A lot of chemopreventive agents act by inhibiting the formation of hydrogen peroxide (Wei and Frenkel 1993, Wei et al 1993). Exner et al (2000) have reported that both salicylate and gentisic acid, have the ability to inhibit LDL oxidation induced by superoxide or by nitric oxide. While some of the phenolic acids like 2,3-DHBA form complex with the metal ions, 2,5-DHBA scavenges the free radicals. Thus, gentisic acid suppresses promoter-mediated oxidative insult in mouse epidermis. It is believed that for chemoprevention of cancer, inhibition of tumor promotion is a better strategy as compared to tumor initiation. This is because tumor initiation is short and irreversible event whereas tumor promotion is a long process wherein many biochemical and histological alterations occur that are reversible during early phases (Zhao et al 1999). It is a well-known fact that oxidants are believed to have a crucial role in all the stages of cancer. Many modulators in other studies and even in our own previous lab studies (Saleem et al 2001, Sultana et al 2003) have shown that agents, which have the ability to prevent oxidative stress are also capable of inhibiting tumor promotion related biochemical alterations e.g. ODC activity. With this view in mind, the compound, gentisic acid was further tested for its anti-tumor promoting ability against TPA and the combination of BPO with UVB. Second mechanism of action that was analyzed for the study of gentisic acid was the inhibition of ODC activity. Induction of ODC activity is considered to be an important biochemical marker of tumor promotion as it is associated with cell proliferation. The data of the current study does not seem to support the role of inhibition of polyamine biosynthetic pathway by the compound. Though gentisic acid appears to have
modulated or suppressed the ODC activity, the suppression was not dose dependent. It suggests that gentisic acid does not have a specific effect on the modulation of ODC activity induced either by TPA or by the combination of BPO and UVB especially not by this mode of action. Surprisingly though, it caused a dose dependent inhibition of unscheduled DNA synthesis. Enkvetchakul et al have also reported some paradoxical facts about another compound, sphingosine, which exhibits inhibitory effect on ODC activity induced by TPA in one study while in the other study, it enhances TPA-promoted tumorigenesis (Enkvetchakul et al 1989, Enkvetchakul et al 1992). There are certain other agents also that do not work by this mechanism of anti-tumor promotion action but are potent anti-tumor agents (Wei et al 1998) although a positive role played by any agent in the amelioration of this marker parameter invariably acts as a potential anti-tumor agent. Various monocyclic phenolic compounds (especially BHA and BHT) have been reviewed for their anticarcinogenic activity (Williams et al 2002) and the compound, gentisic acid also belongs to the category of monophenolic compounds.

The present study also gives an insight into the mechanism of action of Hibiscus rosa sinensis extract against cancer since both the H. sinensis and its chemical constituent, gentisic acid were able to inhibit TPA, BPO and UVB induced unscheduled DNA synthesis. The unscheduled DNA synthesis was dose dependently inhibited by gentisic acid. On the other hand, when we compare the recovery in unscheduled DNA synthesis in case of H. sinensis extract (Chapter 5), we observe that H. sinensis showed more pronounced prevention as compared to that caused by gentisic acid. This suggests that besides gentisic acid other components too have a crucial role in anti-tumor promoting activity of H. sinensis extract. This fact is further strengthened by observations that H. sinensis extract causes dose dependent inhibition of the ODC, the rate-
limiting enzyme in tumor promotion. However, with gentisic acid there surely was statistically significant inhibition of ODC activity but the results obtained were not dose dependent, therefore, we cannot say that gentisic acid has a key role in protection against tumor promotion response via this mechanism. Since oxidative stress is implicated in the etiology of many diseases including initiation, promotion and progression, we can say that gentisic acid might have the ability to interfere with the process of carcinogenesis as an antioxidant. However, owing to its anti-oxidant properties it must have some indirect role in the disease prevention process. Since gentisic acid has sufficient anti-oxidant activity and is also shown to possess inhibitory activities against mutagenesis and cyclooxygenase activity it might play some role in the suppression of inflammation and might have a role on mutations as cooperation of genetic defects and sustained high levels of ODC and polyamine levels are thought to be required for neoplastic transformation and clonal expansion of tumor cells. Different kinds of herbs (Actinidia chinensis, Artemisia lavendulaefolia, Crotalaria sessiflora, Prunella vulgaris, Paris polyphylla and Ampelopsis brevipedunculata) that are used as crude medicine for cancer have been shown to possess antimutagenic potential (Lee and Lin 1988).

The fact that gentisic acid protects against oxidative stress, diminishes incorporation of \(^{3}H\) thymidine into DNA, proves that it has some modulatory activity but since it was unable to dose-dependently inhibit ODC activity, we cannot say that it has key role in protection against tumor promotion. Also, to the best of our knowledge this compound has not been tested against cancer formation. We can nevertheless, conclude that further studies employing different aspects or mechanisms of chemopreventive action should be done to find out whether it has any other mode of action for the prevention of tumor promotion or it has no role in this
preventable stage of tumor formation. Thus, there is a long way to go before any conclusion can be drawn regarding the chemopreventive potential of gentisic acid.
FOOTNOTES AND FIGURE LEGENDS

Figure 1
Role of gentisic acid in BPO and UVB mediated depletion of cutaneous (a) reduced glutathione content and (b) glutathione reductase

Each value represents mean ± SE of six animals. ### p< 0.001 as compared to vehicle treated control group. *** p< 0.001 and ** p< 0.01 when compared with BPO treated and UVB irradiated group. D-1 and D-2 represent topical applications of 2.0 and 4.0 µg gentisic acid/ 0.2ml vehicle / animal.

Figure 2
Modulation of BPO and UVB depleted (a) glutathione-S- transferase and (b) quinone reductase by gentisic acid in mouse skin

Each value represents mean ± SE of six animals. ### p< 0.001, ## p< 0.01 and # p< 0.05 as compared to vehicle treated control group. ** p< 0.01 and * p< 0.05 when compared with BPO treated and UVB irradiated group. D-1 and D-2 represent topical applications of 2.0 and 4.0 µg gentisic acid/ 0.2ml vehicle / animal.

Figure 3
Effect of pretreatment with gentisic acid on BPO and UVB mediated depletion of (a) catalase, (b) glutathione peroxidase and (c) glucose-6-phosphate dehydrogenase in skin

Each value represents mean ± SE of six animals. ### p< 0.001, ## p< 0.01 and # p< 0.05 as compared to vehicle treated control group. ** p< 0.01 and * p< 0.05 when compared with BPO treated and UVB irradiated group. D-1 and D-2 represent topical applications of 2.0 and 4.0 µg gentisic acid/ 0.2ml vehicle / animal.

Figure 4
Effect of pretreatment with gentisic acid on BPO and UVB induced (a) hydrogen peroxide formation and (b) lipid peroxidation in murine skin

Each value represents mean ± SE of six animals. ### p< 0.001 and # p< 0.05 as compared to vehicle treated control group. ** p< 0.01 and * p< 0.05 when compared with BPO treated and UVB irradiated group. D-1 and D-2 represent topical applications of 2.0 and 4.0 µg gentisic acid/ 0.2ml vehicle / animal.

Figure 5
Study of the effect of gentisic acid in the attenuation of BPO and UVB induced cutaneous ODC activity and DNA synthesis

Each value represents mean ± SE of six animals. ### p< 0.001 and # p< 0.05 as compared to vehicle treated control group. *** p< 0.001, ** p< 0.01 and * p< 0.05 when
compared with BPO treated and UVB irradiated group. D-1 and D-2 represent topical applications of 2.0 and 4.0 µg gentisic acid/0.2ml vehicle/animal.

**Figure 6**
Modulatory effect of gentisic acid on TPA mediated depletion of cutaneous (a) reduced glutathione content (b) glutathione-S-transferase and (c) glutathione reductase

Each value represents mean ± SE of six animals. ### p< 0.001 and ## p< 0.01 as compared to vehicle treated control group. *** p< 0.001, ** p< 0.01 and * p< 0.05 when compared with TPA treated group. D-1 and D-2 represent topical applications of 2.0 and 4.0 µg gentisic acid/0.2ml vehicle/animal.

**Figure 7**
Pretreatment effect of gentisic acid on TPA mediated depletion of (a) catalase, (b) glutathione peroxidase and (c) glucose-6-phosphate dehydrogenase in skin

Each value represents mean ± SE of six animals. ### p< 0.001 and # p< 0.05 as compared to vehicle treated control group. *** p< 0.001, ** p< 0.01 and * p< 0.05 when compared with TPA treated group. D-1 and D-2 represent topical applications of 2.0 and 4.0 µg gentisic acid/0.2ml vehicle/animal.

**Figure 8**
Effect of gentisic acid pretreatment on TPA induced (a) hydrogen peroxide formation and (b) lipid peroxidation in mouse skin

Each value represents mean ± SE of six animals. ### p< 0.001 as compared to vehicle treated control group. ** p< 0.01 and * p< 0.05 when compared with TPA treated group. D-1 and D-2 represent topical applications of 2.0 and 4.0 µg gentisic acid/0.2ml vehicle/animal.

**Figure 9**
Pretreatment of gentisic acid on TPA induced cutaneous ODC activity and radiolabelled thymidine incorporation

Each value represents mean ± SE of six animals. ### p< 0.001 when compared with vehicle treated control group. *** p< 0.001 and ** p< 0.01 when compared with TPA treated group. D1 and D2 represent topical applications of 2.0 and 4.0 µg gentisic acid/0.2ml vehicle/animal.
Figure 1
Role of gentisic acid in BPO and UVB mediated depletion of cutaneous (a) reduced glutathione content and (b) glutathione reductase

(a) n mol GSH/gm tissue

(b) nmol NADPH oxidized/min/mg protein

Legend:
- □ Only Vehicle
- □ Only BPO
- □ Gentisic acid (D1)+BPO+UVB
- □ Gentisic acid (D2)+BPO+UVB
- □ Only Gentisic acid (D2)

Treatment groups:
1. Only Vehicle
2. Only BPO
3. Gentisic acid (D1)+BPO+UVB
4. Gentisic acid (D2)+BPO+UVB
5. Only Gentisic acid (D2)
6. BPO+UVB
7. Only UVB
Figure 2
Modulation of BPO and UVB depleted (a) glutathione- S- transferase and (b) quinone reductase by gentisic acid in mouse skin.
**Figure 3**

Effect of pretreatment with gentisic acid on BPO and UVB mediated depletion of (a) catalase, (b) glutathione peroxidase and (c) glucose-6-phosphate dehydrogenase in skin

(a) **nmol H$_2$O$_2$ consumed/min/mg protein**

(b) **nmol NADPH oxidized/min/mg protein**

(c) **nmol NADP reduced/min/mg protein**

Legend:
- □ Only Vehicle
- □ Only BPO
- □ Only UVB
- □ Gentisic acid (D1)+BPO+UVB
- □ Gentisic acid (D2)+BPO+UVB
- □ Only Gentisic acid (D2)
- □ Only UVB
- □ BPO+UVB
**Figure 4**

Effect of pretreatment with gentisic acid on BPO and UVB induced (a) hydrogen peroxide formation and (b) lipid peroxidation in murine skin.

(a) mmol H2O2/gm tissue

(b) mmol of malondialdehyde formed/hr/gm tissue

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<tr>
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<td>Only Gentisic acid (D2)</td>
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</table>
Figure 5
Study of the effect of gentisic acid in the attenuation of BPO and UVB induced cutaneous ODC activity and DNA synthesis.
Figure 6
Moulatory effect of gentisic acid on TPA mediated
depletion of cutaneous (a) reduced glutathione
content (b) glutathione-S-transferase and (c)
glutathione reductase

(a) n mol GSH/gm tissue

(b) nmol CNB-conjugate formed/min/mg protein

(c) nmol NADPH oxidized/min/mg protein

Treatment groups

- Only Vehicle
- Gentisic acid (D1) + TPA
- Only TPA
- Gentisic acid (D2) + TPA
- Only Gentisic acid (D2)
Figure 7

Pretreatment effect of gentisic acid on TPA mediated depletion of (a) catalase, (b) glutathione peroxidase and (c) glucose-6-phosphate dehydrogenase in skin

(a) **nanoM H$_2$O$_2$ consumed/min/mg protein**

(b) **nanoM NADPH oxidized/min/mg protein**

(c) **nanoM NADP reduced/min/mg protein**

Legend:
- **Only Vehicle**
- **Gentisic acid (D1) + TPA**
- **Gentisic acid (D2) + TPA**
- **Only Gentiisic acid (D2)**
Figure 8
Effect of gentisic acid pretreatment on TPA induced (a) hydrogen peroxide formation and (b) lipid peroxidation in mouse skin.

(a) nmol H_2O_2 / gm tissue

(b) nmol of malondialdehyde formed / hr / gm tissue

Treatment groups:
- Only Vehicle
- Gentisic acid (D1) + TPA
- Only Gentisic acid (D2)
- Only TPA
- Gentisic acid (D2) + TPA
Figure 9
Pretreatment of gentisic acid on TPA induced cutaneous ODC activity and DNA synthesis

![Graph 1: ODC activity](image1)

![Graph 2: Thymidine incorporation](image2)

Legend:
- □ Only Vehicle
- □ Gentisic acid (D1) + TPA
- □ Gentisic acid (D2)
- □ Only TPA
- □ Gentisic acid (D2) + TPA