According to the multihit theory for the monoclonal origin of cancer, neoplasia arises when the stem cells of the human tissues that are the random targets of an undetermined number and sequence of inherited, acquired or spontaneous cell-damaging events accumulate nonlethal, unrepaired, irreversible, defects that can be transmitted to successive generations of daughter cells. Genetic and somatic mutations, as well as epigenetic alterations, may result in neoplastic transformation.

The presence of irreversible DNA damage or altered proto-oncogenes increases the probability that the initiated cell might not respond to the regulatory signals for normal growth and differentiation and after neoplastic transformation and a postulated period of dormancy, might then proliferate into a benign or continually the level of DNA lesions/repair in individual stem cells, it appears difficult to predict if and when such irreversible initiation and transformation of a single precursor cell might occur and therefore, to determine at the cellular level when carcinogenesis really begins. (Perchellet et al. 1989)

Chemical carcinogenesis in murine skin is a stepwise process consisting of a series of distinct stages known as initiation, promotion and malignant conversion. There is substantial evidence to implicate the involvement of free radicals, particularly those derived from molecular oxygen in all three stages of chemical carcinogenesis. Since free radicals are known to cause DNA damage, an essential obligatory step in tumor initiation, they are thought to play an important role in this process. However, the role of ROS in tumor initiation is poorly understood.

The involvement of ROS in tumor promotion is suggested by several evidence. In general, tumor promoters increase the generation and decrease the degradation of ROS. Certain organic peroxides and free radical-generating systems exert tumor-promoting activities and enhance certain molecular events related to tumor promotion. Various antioxidants and free radical scavengers inhibit the biochemical and biological effects of
tumor promoters. The application of tumor promoters to murine skin causes significant depletion of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase and catalase. Since tumor promoters are not known to be mutagenic and do not bind to DNA, their effects on aneuploidy, chromosomal aberration, sister chromatid exchange, DNA strand breakage and gene amplification have been thought to be mediated through ROS generated in phorbol ester-treated epidermal cells and released by activated phagocytic cells infiltrating the skin during inflammation.

There is gathering evidence that ROS are involved in the malignant conversion of benign papillomas to carcinomas. The oxidant tumor promoter, benzoyl peroxide, which is a free radical generating compound, has been shown to enhance this process. The fact that cutaneous chemical carcinogens and tumor promoters may, directly or indirectly, generate ROS and that various antioxidants effectively inhibit biochemical and biological events associated with tumor initiation, promotion and malignant conversion, it is conceivable that ROS induced damage in macromolecules may contribute to the transition of epidermal target cells from a preneoplastic state to malignancy. (Athar et al 1991)

There is an increasing awareness that the induction of cancer is a multistep process requiring both initiating and promoting substances for the development of a malignant tumor. In murine skin, tumors are generally induced by a single topical application of the promoting agent. This is known as the two stage carcinogenesis. 7, 12-dimethylbenz(a)anthracene (DMBA), a synthetic PAH, is a conventional agent employed in the majority of studies on murine skin carcinogenesis. The initiation phase of cutaneous chemical carcinogenesis in a two-stage carcinogenesis protocol can be achieved by a single topical application of subthreshold doses of a carcinogen such as DMBA. This is essentially an irreversible step. This subthreshold dose of carcinogen does not produce tumors over the life span of mice. Promotion with multiple applications of croton oil or a phorbol ester such as 12-O-tertadecanoyl-phorbol-13-acetate (TPA), however, leads to the development of benign papillomas after a short latency period. The majority of the benign papillomas thus formed regress, whereas only a few of them develop to carcinoma. Unlike initiation, the promotion phase is initially
reversible but becomes irreversible during later stages. Tumor promoters are usually non-mutagenic and non-carcinogenic agents and application of them alone to normal mouse skin does not produce any tumors. A single topical application of a large dose of carcinogen on mouse skin can also result in cutaneous tumor induction. The latency period however, becomes longer and the number of papillomas produced is lower compared with that produced in initiation promotion protocol. (Mukhtar et al 1989)

Oncogenes are genes that all normal cells contain and in their natural states are known as protooncogenes. Their expression is, sometimes, a part of the normal cell activity. Protooncogenes are activated by mutation or translocation that may ultimately result in tumor growth or tumorigenesis. The role of oncogenes was first demonstrated by Robert A. Weinberg who showed the transformation of normal cells with the DNA from tumor cells. Since then many specific oncogenes have been identified and isolated.

The oncogene that has been demonstrated in many chemically-induced skin tumors are mutated Harvey ras gene (ras$^{ha}$). This gene is a member of a multigene family, present in the genome of all multicellular organisms. All the members of this gene family code for a closely-related membrane bound 21 proteins called p$^{21}$. This protein binds guanosine triphosphatase and has an intrinsic guanosine triphosphatase activity. The activation of ras$^{ha}$ in skin appears to be mediated by a point mutation in the cellular oncogene. The product of mutated oncogene p$^{21}$ is altered by one amino acid in which guanosine triphosphate activity is reduced. Similar results have been obtained in different studies by mutating cloned normal ras$^{ha}$ by the exposure to a skin carcinogen. The mutation of ras$^{ha}$ seems to be an early initiating event, but absence of this activated oncogene in all papillomas and conversion of such a papilloma into carcinoma by the incorporation of cloned activated ras$^{ha}$ oncogene, suggests its role in malignant transformation. Balmain et al demonstrated that the introduction of activated Harvey murine sarcoma virus ras genes into epidermal cells of mouse skin in vivo followed by treatment with tpa-induced benign papillomas progressed to invasive carcinoma. This is quite similar to that of the two-stage initiation promotion protocol. The event of activation of ras$^{ha}$
does not, however, seem to be sufficient for oncogenic action unless it is coupled with other important events which is not clearly defined.

Aloin still has an important role in the traditional medicine of many contemporary cultures. In India, Aloin medications are used for a variety of conditions, particularly for their cathartic, stomachic, emmengogic and anthelminthic properties (Chopra et al., 1956). Whole leaves, the exudates, and the fresh gel are all used. In China Aloin has been an important medicine for centuries, and it is still a common household remedy (Cole and Chen, 1943). In Mexico, the leaves are gathered from plants and growing semi-wild to treat burns, bruises, skin irritations, and even leprosy. Aloin is also widely used as a folk remedy across the rest of Middle America and the West Indies, as reviewed by Morton (1981). In all these countries Aloin is an introduced species, but has been rapidly adopted as an essential part of local material medica.

In present day Western Society, most notably in the USA, Aloin has fairly frequent use in homeopathy and herbalism (Panos and Heimlich, 1980). It is commonly grown in America and the tropics as a pot plant on kitchen windowsills, so the leaves are on hand to treat burns, to soothe the pain and promote healing. The plant has additional use to treat sun burn and various dermatological conditions and taken internally, as a general tonic. Madis Laboratories (1984) list over a hundred medical disorders that have at some time been treated, such as arthritis, gout, ache, cuts, dermatitis, headache, high blood pressure, indigestion, hair loss, rheumatism, peptic ulcers, mouth diseases, pruritis, psoriasis and, of course, burns. The medicinal use of Aloin is particularly widespread in Florida (Galban, 1952). Some of the more unusual applications include bee stings. Aloin has also had considerable use as a folk remedy for farm animals, as described by Anderson (1983). Among these pharmacological effects, the antimeoplastic effect of Aloin is of interest since a number of polysaccharides have been studied for antitum originiaty (Koboyaski et al., 1993). Thus, Aloin is worthy of investigation as a chemopreventive agent.

In spite of the progress made by many investigators in the last several years to elucidate the mechanism by which food flavonoids exert their protective effects in reducing carcinogenicity, the precise mechanism of
action is still unclear (Stich, 1991). Studies to identify agents (principles) in food with anticarcinogenic potential, have shown that most of these compounds (e.g. Vitamins c, e, a beta-carotene, selenium) possess antioxidative potential (Amstad et al., 1990). Since many flavonoids are also antioxidants such as hesperidin, it is likely that the antioxidative property of flavonoids is, at least in part, responsible for their reported anticarcinogenic potential.

We, in this study, have proved the inhibitory potential of aloin and hesperidin against DMBA-induced initiation of tumorigenesis in Swiss albino mice in a two-stage initiation-promotion tumor development model. The results of studies described in this chapter suggest that these compounds possess strong anti-tumor initiating and promoting potential in the model studied.

**EXPERIMENTAL PROTOCOL**

**TREATMENT OF ANIMALS FOR CUTANEOUS TUMOR STUDIES**

The dorsal skin of female Swiss albino mice was shaved and mice that were at the resting phase of hair cycle were used for skin tumor induction studies. The dorsal shaved skin of the mice was initiated with a single topical application of DMBA (40μg in 150μl acetone), under subdued light. One week after initiation, all of the mice were treated with topical twice-weekly applications of TPA (2.5μg in 200μl acetone) for twenty weeks.

**ANTIPROMOTION STUDIES WHEN ALOIN AND HESPERIDIN WAS ADMINISTERED ORALLY**

Female Swiss albino mice that were at the resting phase of hair cycle were divided into six groups of thirty mice each.

- **Group-I** - Acetone
- **Group-II** - DMBA+TPA (as mentioned above).
- **Group- III and IV** - DMBA+TPA and Aloin (Orally given, at a dose level of 100 mg/kg body weight and 300 mg/kg body weight respectively).
- **Group- V and VI** - DMBA+TPA and Hesperidin (Orally given, at a dose level of 25 mg/kg body weight and 50 mg/kg body weight respectively).
These treatments were continued until the termination of the experiment and the numbers of papillomas were counted weekly. The data are expressed as the percentage of mice with papillomas and the number of papillomas/mouse and are plotted as function of weeks on test.

ANTI-INITIATION STUDIES WHEN ALOIN AND HESPERIDIN WAS ADMINISTERED ORALLY

Group distribution and the treatment protocol is the same as described above except that treatment of aloin and hesperidin was given two month prior to DMBA+TPA application.

TREATMENT OF ANIMALS FOR BIOCHEMICAL ESTIMATIONS.

In this study, eight groups of mice consisting of six animals each were used.

Group-I - Acetone
Group-II - DMBA+TPA (2.5μg/mouse).
Group-III - Higher dose of Aloin (300 mg/kg body weight).
Group-IV and V - DMBA+TPA and Aloin (Orally given, at a dose level of 100 mg/kg body weight (A1D) and 300 mg/kg body weight (A2D) respectively).
Group-VI - Higher dose of hesperidin alone (50 mg/kg body weight)
Group-VII and VIII - DMBA+TPA and Hesperidin (Orally given, at a dose level of 25 mg/kg body weight (H1D) and 50 mg/kg body weight (H2D) respectively).

The animals were killed 12 hrs. after the application of TPA for the assay of antioxidant enzymes and lipid peroxidation, 6 hrs. after the application of TPA for ODC induction studies. For the \[^{3}\text{H}]\text{thymidine}=\text{incorporation studies, 18 hrs after the application of TPA}\ [^{3}\text{H}]\text{thymidine was injected intraperitoneally to animals. These animals were then sacrificed 2 hrs. after the [^{3}\text{H}]\text{thymidine treatment.}
RESULTS

EFFECT OF ORAL PRETREATMENT OF ALOIN AND HESPERIDIN ON TPA-MEDIATED CUTANEOUS TUMOR PROMOTION:

The effect of oral pre-treatment of animals with aloin and hesperidin on the TPA-mediated tumor promotion in DMBA-initiated mice is shown in Figure-1. Pre-treatment of animals with hesperidin and aloin reduced the tumor promoting effect of TPA as evidenced by the decrease in the total number of tumors as well as their incidence observed in these animals. After 20 weeks on test, the number of papillomas/mouse were 5.6 ±0.87 in animals receiving TPA (alone) as compared to 4.55±0.85 (p<0.008), 3.06 ±0.72(p<0.02), 3.75 ± 0.75 (p<0.02) and 2.23±0.94 (p<0.008), in the animals receiving aloin (100 mg/kg body weight and 300 mg/kg body weight) and hesperidin (25 mg/kg body weight and 50 mg/kg body weight) respectively which was statistically significant when compared with TPA alone (positive control) group. The tumor incidence which was 100% in TPA-treated group, reduced to 64%, 60%(p<0.01), 60%(p<0.09) and 56%(p<0.0006) in groups receiving aloin (100 mg/kg body weight and 300 mg/kg body weight) and hesperidin (25 mg/kg body weight and 50 mg/kg body weight) respectively this was also statistically significant when compared with TPA alone (positive control) group.

EFFECT OF ORAL PRETREATMENT OF ALOIN AND HESPERIDIN ON CUTANEOUS TUMOR INITIATION

The effect of oral pre-treatment of aloin and hesperidin on tumor initiation is shown in Figure-2. Pre-treatment of animals with hesperidin before initiating with DMBA resulted in a decrease in the total number of tumors as well as their incidence observed in these animals. After 20 weeks on test, the number of papillomas/mouse were 5.2 ±1.27 in animals receiving TPA (alone) as compared to 4.74 ±0.88(0.06), 3.49±0.64(p<0.04), 3.98 ±0.95(p<0.01), and 2.45 ±0.86(p<0.02) in the animals receiving aloin (100 mg/kg body weight and 300 mg/kg body weight) and hesperidin (25 mg/kg body weight and 50 mg/kg body weight) respectively which was statistically significant when compared with TPA alone (positive control)
EFFECT OF PRETREATMENT OF ALOIN AND HESPERIDIN ON TPA MEDIATED EPIDERMAL ODC INDUCTION AND ENHANCEMENT OF $[^3]H$ THYMIDINE INCORPORATION

The effect of pre-treatment of aloin and hesperidin on TPA-mediated induction of epidermal ODC activity and enhancement of $[^3]H$ thymidine incorporation is given in Table-1. A inhibitory effect of hesperidin and aloin on TPA-mediated induction of ODC activity was observed. TPA (alone) treatment induced the ODC activity by 1200% of the acetone-treated control. The pre-treatment of aloin reduced the TPA-mediated ODC to about 1046% (p<0.05) & 939% (p<0.01) of the acetone-treated control but pre-treatment of hesperidin reduced the TPA-mediated ODC induction to about 960% (p<0.05) & 895% (p<0.001) of acetone-treated control.

TPA (alone) treatment resulted in about 316% enhancement in the incorporation of $[^3]H$ thymidine in epidermal DNA as compared to acetone-treated control. However, in aloin and hesperidin treated animals, this enhancement was significantly reduced as compared to TPA (alone) treatment group. aloin resulted in 278% (p<0.05) & 244% (p<0.05) reduction in $[^3]H$ thymidine incorporation, whereas hesperidin resulted in a 244% (p<0.05) & 214% (p<0.001) reduction in $[^3]H$ thymidine incorporation, in cutaneous DNA.

EFFECT OF ALOIN AND HESPERIDIN ON CUTANEOUS XANTHINE OXIDASE AND MICROSOMAL LPO

The increase in XO activity was found to be in a dose dependent manner as shown in fig. 3. The increase in XO activity was 67.73% (p<0.01) and 75.19% (p<0.01) in mice, which were orally fed on aloin 100 mg/kg body weight and 300 mg/kg body weight respectively. Similarly, increase in
XO activity was 80.24% (p<0.001) and 84.5% (p<0.05) in mice, which were orally fed on hesperidin 25 mg/kg body weight and 50 mg/kg body weight respectively. All the values was statistically significant when compared with TPA alone group.

Similarly, a decrease in microsomal LPO was found 101.31% (p<0.001) and 104.52% (p<0.05) in the skin of mice, which were fed on aloin 100 mg/kg body weight and 300 mg/kg body weight respectively. Similarly, a decrease in microsomal LPO was found 102.65% (p<0.05) and 107.53% (p<0.01) in the skin of mice, which were fed on hesperidin (25 mg/kg body weight and 50 mg/kg body weight) respectively. All the values was statistically significant when compared with TPA alone group.

EFFECT OF PRETREATMENT OF ALOIN AND HESPERIDIN ON TPA-MEDIATED EPIDERMAL LEVELS OF GSH AND GSH-METABOLIZING AND ANTIOXIDANT ENZYMES

The effect of pre-treatment of mice with aloin and hesperidin on TPA-mediated depletion in the levels of epidermal GSH and on the activities of various GSH metabolising enzymes viz. GR, GST and antioxidant enzymes GPx and catalase, is shown in Fig- 4, 5 & 6. TPA (alone) treatment resulted in the depletion of epidermal GSH and decrease in the activities of GR and GST to 60.23% (p<0.001), 52.17% (p<0.01) and 60.13% (p<0.001) respectively of the corresponding acetone-treated control value. The pre-treatment with hesperidin and aloin resulted in the significant recovery of the TPA-mediated depletion in activities of these enzymes. With aloin the recovery ranged from 16 to 20% of the acetone-treated control group whereas with hesperidin, this recovery ranged from 10 to 18% of the acetone-treated control.

TPA-treatment results in the depletion of activities of GPx and catalase to a level of 59.37% and 56.07% (p<0.01) of acetone-treated control value. The pre-treatment with aloin ranged from 16 to 24% of the acetone-treated control group whereas with hesperidin resulted in recovery, which ranged from 10 to 14% of the acetone-treated control group.
DISCUSSION

ROS have a ubiquitous presence and may be encountered via diet, drugs or environmental pollutants. Entry of ROS generating substances in the body predisposes various cells/tissue/organ to oxidative stress, which may alter their physiology. Various processes in the body may also lead to formation of ROS. ROS, whether of exogenous or endogenous origin, are capable of reacting with almost all cellular macromolecules and may profoundly alter physiology of the cell (Sagara et al., 1998). Topical application of TPA has been reported to increase release of free radicals. Many tumor promoters have been shown to exert their action by production of reactive oxygen species (ROS) and many compounds that possess antioxidant activity have been reported to inhibit tumor promotion (Perchellet & Perchellet, 1989; Sun, 1990). Furthermore, detoxification protects cells from a wide variety of carcinogens and endogenous tumors. Phase I enzymes including cytochrome P450 metabolically activate carcinogens to generate products which are highly reactive electrophiles (i.e. epoxides and reactive oxygen species). In contrast, phase II detoxification enzymes both complete with the phase I activating enzymes to inhibit the formation of electrophile and catalyze the conversion of electrophile to inactive conjugates, making them more water soluble and more readily excreted from the cells (Khan et al. 1992; Wattenberg, 1992). Induction of phase II enzymes by naturally occurring or synthetic agents represents a promising strategy for cancer prevention (Bertram et al. 1987; Wattenberg, 1992). Tumor promotion is known to involve oxidative stress at least during early stages. Therefore, most of the tumor promoters have been shown to act through the elaboration of pro-oxidant response (Athar et al., 1991). TPA a phorbol ester type tumor promoter has been shown to induce oxidative stress in murine skin by the generation of free radicals (Slaga et al., 1984). In addition, TPA treatment resulted in the depletion of the activity of all the major cutaneous antioxidant enzymes and other non-enzymatic antioxidant molecules. Pre treatment of aloin and hesperidin to TPA- treated mice ameliorated TPA mediated oxidative stress, which is evident by the attenuation in the level of glutathione and increase in the activities of all major antioxidant enzymes, suggesting the potential role of aloin and
hesperidin, which is rich in antioxidant in alleviating TPA mediated cutaneous oxidative stress.

Incomplete reduction of the molecular oxygen by a single electron causes the production of FOR. It has been demonstrated that FOR play a role in cytotoxicity and carcinogenesis as promoter and initiator. They are known to cause mutations, sister chromatid exchanges and chromosomal deletions. It has been found that tumor cells have only a decreased amount of mitochondrial superoxide dismutase, which is an endogenous FOR scavenger enzyme. (Kilic et al 1986)

The effect of chemopreventive agents have been related to their ability to enhance the activities of carcinogen metabolizing enzymes and/or to bind with them thus reducing their effective critical tissue concentrations required for the manifestation of carcinogenesis. Their antioxidant action counteracts the increased amount of oxygen radicals generated by chemicals during the course of their metabolism (Wattenberg 1985). These oxygen radicals have been implicated to be involved in various stages of carcinogenesis (Athar et al. 1992; Giri et al. 1995, 1996). For the prevention of toxic effects of various mutagens and carcinogens (Wattenberg 1983) the dietary intake of natural antioxidants have been suggested. The interception caused by these agents may be at different stages of the multi-step process of carcinogenesis (Perchellet and Perchellet 1989). Many of these agents have properties to other more than one event involved in these steps of carcinogenesis. Therefore, the pre-treatment with these agents often inhibits more than one step of carcinogenesis. Therefore, the pre-treatment with these agents often inhibits more than one step of carcinogenesis. Their anticarcinogenic activity has been attributed to antioxidant potentials of these compounds.

Tumor cell biochemistry can be affected positively by the inhibition of promoting, if radical scavenger agents are administered exogenously. Some flavonoids and aloes have been reported to possess biological properties, e.g. antitumor, antibacterial, antiviral, antimutagenic and antioxidative properties. Some of these properties derive from the free radical scavenging activities of flavonoids and aloes. There are relating reactivities of flavonoids and aloes with active oxygen species. The relation can be find between
The antioxidative ability of lipid peroxidation and OH⁻ scavenging effect of flavonoid in spite of general recognition that lipid peroxidation is initiated by OH⁻. A flavonoid hesperidin and aloe aloin has also been reported to have preventive effects on skin inflammation by the way of both scavenging FOR and initiating pre-inflammatory mediators such as prostaglandin, which induce neutrophil chemotaxis. (Lonchampt et al 1989)

Another possible explanation for the effect of hesperidin may involve the electron transfer reactions in aerobic respiratory systems. Any degeneracy in the electron transfer chain that supplies the aerobic system in the inner membrane of mitochondria of a cell causes the failure of electron transfer of oxygen. In this way the cell may be induced to supply mostly anaerobic energy, which may cause mutation in the DNA of the mitochondria and in the nucleus. It is also known that the electron chain is either broken or damaged in most of the cancer cells turn to a glycolysis pathway from aerobic respiration when transformed into malignant phenotype. (Baykut et al 1989)

It has been shown that hesperidin plays a role in the synthesis of ascorbic acid in nature due to its capacity as a reversible electron transferring substance. The specific potential energy of hesperidin is 0.715-volt (Baykut et al 1987). So, the defect occurring in the electron chain flow in the mitochondria of the cancer cell may be compensated by hesperidin.

TPA which promotes tumor formation and alters the related biochemical events such as increase in protein kinase activity, induction of ornithine decarboxylase activity, enhanced DNA synthesis, increased prostaglandin synthesis, is a agent acting, by generating oxidative stress through the depletion of antioxidant enzymes and enhanced generation of oxygen free radicals.

Hesperidin and aloin are used in our study to assess the efficacy against TPA-mediated tumor promotion in mice skin. The results of this study suggest hesperidin and aloin to be an effective inhibitor of TPA induced increase in epidermal carcinogenesis. These compounds not only inhibits the TPA induced increase in epidermal ODC activities and DNA synthesis, but it also resulted in the reversal of depleted levels of glutathione.
and its metabolising enzymes induced by TPA. Hesperidin and aloin also suppressed the malignant conversion of papillomas to carcinomas.

The study shows that these compounds has an ability to facilitate and enhance the activity of GSH dependant antioxidant protective system of the epidermal cells during the later stages of tumor promotion. These compounds shows the efficacy to scavenge singlet oxygen and thus inhibit the initiation of lipid peroxidation.

The induction of ODC activity is considered to be closely associated with the tumor promoting activity of a variety of tumor promoters. It is involved in the polyamine biosynthesis which plays a major role in cell proliferation and differentiation. The importance of ODC induction in tumor promotion is evident from the fact the several inhibitors of ODC are capable of inhibiting tumor promotion. These compounds effectively inhibit the TPA mediated induction of ODC activity suggesting that they may be acting by inhibiting early biochemical responses of tumor promotion. These compounds also reduces DNA synthesis induced by TPA indicating its ability to reduce the TPA induced mitogenic response on skin (Huachen et al 1998)

This study suggests that flavonoid hesperidin and aloe aloin inhibit the tumor promotion induced by TPA in mouse skin carcinogenesis. Thus, these compounds are potent antitumor promoter in murine skin and acts by inhibiting the oxidative stress. Therefore, the observed inhibition in the development of papillomas in skin of mice receiving a pre-treatment of aloin and hesperidin suggests that the inhibition may be due to the antioxidant components of these compunds.

In summary, our results suggest that aloin and hesperidin is an effective anti-tumor promoter, anti-tumor initiation and delays the cutaneous tumor induction and development. Our results also showed that the aloin and hesperidin is an effective suppressor of malignant tumor growth in murine skin and its acts by inhibiting cutaneous oxidative stress.
Table 1: Effect of oral pretreatment of mice with aloin and hesperidin on TPA-mediated induction of epidermal ODC activity and enhancement of $[^{3}H]$ thymidine incorporation.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>ODC activity $^{14}$CO$_{2}$ released/hr/mg protein</th>
<th>% of control</th>
<th>$[^{3}H]$ thymidine incorporation DPM/μg DNA</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>204.5 ± 10.41</td>
<td>100</td>
<td>320.17 ± 4.8</td>
<td>100</td>
</tr>
<tr>
<td>TPA alone</td>
<td>2427 ± 48.73*</td>
<td>1200</td>
<td>1014.33 ± 5.48**</td>
<td>316</td>
</tr>
<tr>
<td>A2D alone</td>
<td>218.1 ± 13.09**</td>
<td>106</td>
<td>339.17 ± 16.96**</td>
<td>274</td>
</tr>
<tr>
<td>TPA + A1D</td>
<td>2158 ± 28.48**</td>
<td>1046</td>
<td>882.01 ± 11.44*</td>
<td>275</td>
</tr>
<tr>
<td>TPA + A2D</td>
<td>1938 ± 96.9*</td>
<td>939</td>
<td>782.15 ± 15.8*</td>
<td>244</td>
</tr>
<tr>
<td>H2D alone</td>
<td>217.3 ± 10.87**</td>
<td>105</td>
<td>331.17 ± 16.56***</td>
<td>103</td>
</tr>
<tr>
<td>TPA + H1D</td>
<td>1981 ± 11.86*</td>
<td>960</td>
<td>782.17 ± 46.93*</td>
<td>244</td>
</tr>
<tr>
<td>TPA + H2D</td>
<td>1848 ± 92.4***</td>
<td>895</td>
<td>688.11 ± 34.41***</td>
<td>214</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. of six animals.
Significantly different *p<0.05, ** p<0.01 and ***p<0.001.
A1D- aloin 100 mg/kg body weight, A2D - aloin 300 mg/kg body weight
H1D- hesperidin 25 mg/kg body weight, H2D -hesperidin 50 mg/kg body weight.
Fig. 1: Anti-promotion studies of Aloin and Hesperidin on DMBA initiated and TPA-mediated mice skin tumorigenesis.

The number of papillomas/mouse and % incidence of tumors were plotted as a function of number of weeks on test. Dose regimen and treatment protocols are described in the text.

A1D- Aloin 100 mg/kg body weight, A2D- Aloin 300 mg/kg body weight
H1D- Hesperidin 25 mg/kg body weight, H2D- Hesperidin 50 mg/kg body weight.

DMBA- Dimethylbenz(a)anthracene, TPA- 12-O-tetradecanoyl phorbol 13, acetate
Fig. 2: Anti-initiation studies of Hesperidin and Aloin on DMBA initiated and TPA-mediated mice skin tumorigenesis.

The number of papillomas/mouse and % incidence of tumors were plotted as a function of number of weeks on test. Dose regimen and treatment protocols are described in the text.

A1D- Aloin 100 mg/kg body weight, A2D- Aloin 300 mg/kg body weight
H1D- Hesperidin 25 mg/kg body weight, H2D-Hesperidin 50 mg/kg body weight.
DMBA- Dimethylbenz(a)anthracene, TPA- 12-O-tetradecanoyl phorbol 13, acetate
Fig. 3: Effect of Aloin and Hesperidin on TPA-mediated alteration of epidermal Xanthine oxidase and lipid oxidase activity.

Each data represents % of saline treated control value. The values are calculated as mean ± S.E of six animals. Dose regimen and treatment protocols are described in the text.

(*) Significantly different (p<0.05), (**) Significantly different (p<0.01) and (***) Significantly different (p<0.001) when compared with saline treated treated group.

A1D- Aloin 100 mg/kg body weight, A2D- Aloin 300 mg/kg body weight
H1D- Hesperidin 25 mg/kg body weight, H2D- Hesperidin 50 mg/kg body weight.
TPA- 12-O-tetradecanoyl phorbol 13, acetate.
Fig. 4: Effect of Aloin and Hesperidin on TPA-mediated alteration of epidermal glutathione peroxidase and catalase activity.

Each data represents % of saline treated control value. The values are calculated as mean ± S.E of six animals. Dose regimen and treatment protocols are described in the text.

(*) Significantly different (p<0.05), (**) Significantly different (p<0.01) and (***) Significantly different (p<0.001) when compared with saline treated treated group.

A1D- Aloin 100 mg/kg body weight, A2D- Aloin 300 mg/kg body weight
H1D- Hesperidin 25 mg/kg body weight, H2D-Hesperidin 50 mg/kg body weight.
TPA- 12-O-tetradecanoyl phorbol 13, acetate
Fig. 5: Effect of Aloin and Hesperidin on TPA-mediated alteration of epidermal glutathione-S-transferase and glutathione reductase activity.

Each data represents % of saline treated control value. The values are calculated as mean ± S.E of six animals. Dose regimen and treatment protocols are described in the text.

(*) Significantly different (p<0.05), (**) Significantly different (p<0.01) and (***) Significantly different (p<0.001) when compared with saline treated treated group.

A1D - Aloin 100 mg/kg body weight, A2D - Aloin 300 mg/kg body weight
H1D - Hesperidin 25 mg/kg body weight, H2D - Hesperidin 50 mg/kg body weight.
TPA - 12-O-tetradecanoyl phorbol 13, acetate