INHIBITORY EFFECT OF HESPERIDIN AND ALOIN ON FORESTOMACH CARCINOGENESIS

Internationally, the highest rates of stomach cancer are found in Japan, but there are other parts of the world which have high rates. They include South America, China, Iceland and Eastern Europe. The stomach cancer is the commonest of malignant disease. The wall of stomach has four layers, which are, mucosa, submucosa, muscularis mucosa, serosa. The last three layers are similar in both the compartment of stomach i.e. glandular and forestomach (Ali et al 1990).

The mucosa of forestomach is composed of keratinized stratified squamous epithelium without any glands. The mucosa of glandular stomach is lined by a layer of edumna epithelium that forms a fold along with elements of edumna epithelium that forms a fold along with elements of lamina propria. At the folds various types of glands open and liberate their secretion for digestion of food. Glandular stomach consists of three parts, cardiac, fundic and pyloric. Fundic glands are the major parts of glandular stomach having chief cells and parielal cells (Masa- Aki et al 1993).

Cell growth is a carefully regulated process that responds to specific needs of the body, in a young animal, cell multiplication exceeds cell death, so the animal increases in size; in an adult, the processes of cell birth and death are balanced to produce a steady state. For some adult cell types, renewal is rapid. Intestinal cells have a half life of a few days before they die and are replaced as rapidly. Very occasionally, the exquisite controls that regulate cell multiplication break down and a cell begins to grow and divide, although the body has no need for further. Cells for its type. When the descendants of such a cell inherit the propensity to grow without responding to regulation, the result is a clone of cells able to expand indefinitely. Ultimately a mass called a tumor may be formed by this clone of unwanted cells. Because tumors may have devastating effects on the animals that harbour them (Bradshaw et al 1984).
Chemicals were originally associated with cancer through experimental studies in animals. The metabolic activation of carcinogens is carried out by enzymes that are normally resident in the body. Animals have such enzymes, especially in liver, because they are part of a system that detoxifies noxious chemicals that make their way into the body. Therapeutic drugs, insecticides, polycyclic hydrocarbons and some natural products are often so fat-soluble and water insoluble that they would accumulate continually in fat cells and lipid membranes if there were no way for the animal to excrete them. The detoxification system works by solubilization: it adds hydrophilic groups to water-insoluble compounds, thus allowing the body to rid itself of noxious or simply insoluble materials (Melnick et al. 1985).

The detoxification process begins with a powerful series of oxidation reactions catalyzed by a set of proteins called cytochrome P-450s. These enzymes, which are bound to endoplasmic reticulum membranes, can oxidize even highly unreactive compounds such as polycyclic aromatic hydrocarbons. The metabolic activation of benzo(a)pyrene, a polycyclic aromatic hydrocarbon that is a powerful carcinogen. Although chemically almost inert, it becomes a highly reactive electrophile due to metabolic conversions. There are two metabolic pathways. The first pathway involves an intermediate epoxide formed by the attack of the cytochrome P-450 system on the "K region" of the molecule. An epoxide in this region is rapidly hydrolyzed to a nonreactive dihydrodiol. The second pathway involves an initial oxidation at the 7, 8- double bond, leading to a 7, 8-oxide that is rapidly converted to a 7, 8-dihydrodiol. This compound is still a good substrate for the P-450 system and is again epoxidated, now near the "bay region" at the 9, 10 double bond. The 7,8-diol, 9,10-oxide (or diol epoxide) is not a good substrate for epoxide hydratase, so it is released into the cell as a highly reactive electrophile. This form is carcinogenic because it can readily react with negatively charged centers in DNA (Baltimore et al. 1981).

The use of A. vera gel taken internally to treat peptic ulcers was reported by Blitz et al. (1963). Twelve patients diagnosed as suffering
from peptic ulcers, confirmed by X-ray evidence of duodenal lesions, were given an emulsion of A. vera in petrolatum. Complete cures were claimed, even after a period of a year, and they wrote "Usually, such unmistakable lesions are accompanied by exacerbations of distress once and more often twice a year under any form of medical treatment, but no such episodes were experienced in this series of cases". They also reported that X-ray examination showed complete healing. The effect of the A. vera gel was attributed by the writers to coacervation of pepsin, inhibition of hydrochloric acid secretion and a general detoxifying effect. This work has not been followed up by a full clinical trial to the present knowledge of these authors, but Blitz et al. (1963) pointed out that if A. vera was not pharmacologically active "as the indictment of western medicine has intimated" then the observed 100% complete recovery would not be expected, nor would the observed 100% complete recovery not be expected, nor would the cessation of pain at mealtimes which accompanied the A. vera treatment.

Approximately 4000 different flavonoids have been chemically identified in plant extracts worldwide, making those widespread compounds important constituents of the natural human diet. Pharmacological activities have been attributed to some flavonoids, particularly those related to their anti-inflammatory and analgesic properties. Hesperidin may have antioxidant, anti-inflammatory, anti-allergic, hypolipidemic, vasoprotective and anticarcinogenic actions (Galati et al. 1994). Flavonoid curcumin have been repoted by Singh et al. (1998) to cause a significant inhibition in B(a)P induced forestomach carcinogenesis.

However, so far no study has been conducted on the effect of aloin and hesperidin on forestomach carcinogenesis. In the present study we have investigated the anti-initiating and anti-promoting effect of oral supplementation of aloin and hesperidin on benzo[a]pyrene (B(a)P)-induced forestomach carcinogenesis in mice. We have also studied the effect of aloin and hesperidin supplementation on B(a)P induced lipid peroxidation (LPO) and oxidative stress in murine forestomach.
EXPERIMENTAL PROTOCOL

ANIMALS
Six to eight week old female Swiss albino mice were used in this study.

TREATMENT OF ANIMALS FOR TUMOR STUDIES
Forestomach tumors were induced by oral administration of 1mg BP in 200μl corn oil/mouse by gavage twice weekly for six weeks (Silva et al. 2001).

ANTIPROMOTION STUDIES WHEN ALOIN AND HESPERIDIN IS GIVEN ORALLY
Female Swiss albino mice were divided into six groups of thirty mice each. Group-I was given corn oil orally by gavage (twice weekly for six weeks). Group-II was given BP (1 mg/ mouse twice weekly for six weeks). Group-III and IV were given BP 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 were given BP and Hesperidin (Orally given, at a dose level of 25 mg/kg body weight and 50 mg/kg body weight respectively). Hesperidin and aloin were administered one hour before administration of BP.

ANTI-INITIATION STUDIES WHEN ALOIN AND HESPERIDIN IS GIVEN ORALLY
Group distribution and the treatment protocol is the same as described above except that treatment of Hesperidin and aloin was given one month prior to BP treatment.

At the end of 42 weeks animals were killed by cervical dislocation. The stomachs were fixed in an expanded state produced by i.g. injection of 10% buffered formalin and split longitudinally, and tumors of the forestomach were counted under a dissecting microscope. Tumors 1mm
or longer were recorded and verified histopathologically (Wattenberg 1978). Forestomach were removed and preserved in 10% buffered formalin for histopathological studies. Hematoxylin and eosin preparations of processed sections were prepared for microscopic examination.

TREATMENT OF ANIMALS FOR OXIDATIVE STRESS

For oxidative stress parameters additional groups of animals were used. For studying the effect of aloin and hesperidin on BP mediated generation of forestomach oxidative stress, female Swiss albino mice were taken which were divided into 8 groups of 6 mice in each.

Group-I - given corn oil orally by gavage.
Group-II - given BP (1 mg/200μl /mice).
Group-III – Higher dose of Aloin (300 mg/kg body weight).
Group-IV and V - BP (1 mg/ 200μl / mice) 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 - BP (1 mg/ 200μl / mice) and Hesperidin (Orally given, at a dose level of 25 mg/kg body weight (H1D) and 50 mg/kg body weight (H2D) respectively).

All the animals were sacrificed 24 hours after the last dose of aloin and hesperidin or BP by cervical dislocation within a short span period of one hour.

TISSUE PREPARATION FOR BIOCHEMICAL ASSAYS

After killing the mice the animals were immediately dissected to remove the forestomach, which was washed in ice-cold saline (0.9%) to remove extraneous materials. All subsequent operations were carried out on ice at a temperature 4°C. The washed tissue was blotted between the folds of a filter paper and weighed on a balance (Sartorius, BP 210 D). For biochemical studies, a known amount of tissue was taken and homogenized in chilled phosphate buffer (0.1M, pH 7.4). The forestomach
homogenate was centrifuged at 9000g for 20min to get the post mitochondrial supernatant (PMS), which was used for the estimation of GSH, GPx, GR, GST, G-6-PD, catalase and LPO.

RESULTS

EFFECT OF ORAL FEEDING OF ALOIN AND HESPERIDIN ON BENZ(A)PYRENE-MEDIATED FORESTOMACH TUMOR PROMOTION

Oral supplementation of aloin and hesperidin resulted in a decrease in the number and incidence of forestomach tumors induced by oral administration of 1mg BP (Table-1). BP treatment resulted in 3.25±0.60 (P<0.05) number of tumors and 100% incidence of tumors. When mice were treated with aloin the number and % incidence of tumors was 1.95±0.24(P<0.05) and 60% in mice which received aloin at a dose level of 100 mg/kg body weight and 1.85±0.18 (P<0.01) in mice which received aloin at a dose level of 300 mg/kg body weight at the promotion stage of forestomach carcinogenesis along with BP treatment. Whereas the number and % incidence of tumors was 1.79±0.60 (P<0.01) and 50% in mice which received hesperidin at a dose level of 25 mg/kg body weight and 1.39±0.37(P<0.01) in mice which received hesperidin at a dose level of 50 mg/kg body weight at the promotion stage of forestomach carcinogenesis along with BP treatment.

EFFECT OF ORAL FEEDING OF ALOIN AND HESPERIDIN ON BENZO(A)PYRENE-MEDIATED FORESTOMACH TUMOR INITIATION

Oral supplementation of aloin and hesperidin given one month prior to B(a)P treatment resulted in a decrease in the number and incidence of forestomach tumors induced by oral administration of 1mg B(a)P (Table-2). BP treatment resulted in 4.8±0.20 (P<0.01) number of tumors and 100% incidence of tumors. When mice were treated with aloin the number and % incidence of tumors was 3.02±0.30 (P<0.01) and 63% in mice which received aloin at a dose level of 100 mg/kg body weight and 2.88±0.36 (P<0.05) and 55% in mice which received aloin at a dose level of 300
mg/kg body weight at the promotion stage of forestomach carcinogenesis along with B(a)P treatment. Whereas the number and % incidence of tumors was 2.4±0.24 (P<0.05) and 60% in mice which received hesperidin at a dose level of 25 mg/kg body weight and 2.16±0.29 (P<0.001) in mice which received hesperidin at a dose level of 50 mg/kg body weight at the promotion stage of forestomach carcinogenesis along with BP treatment.

**EFFECT OF ALOIN AND HESPERIDIN ON FORESTOMACH XANTHINE OXIDASE AND MICROSOMAL LPO**

Effect of pretreatment of mice with aloin and hesperidin on benzo(a)pyrene mediated forestomach levels of xanthine oxidase. Benz(a)pyrene (alone)-treatment diminished the levels of XO by 56.23% (P<0.01). The increase in XO activity was found to be in a dose dependent manner as shown in fig. 1. The increase in XO activity was in the range of 67.73 to 82.3% in mice, which were orally fed on 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). A increase in the higher doses only, of aloin and hesperidin were observed about 121.61% (P<0.01) and 132% (P<0.01) respectively when compared to control group.

Similarly, a decrease in LPO activity was found to be in a dose dependent manner as shown in fig. 1. The increase in LPO was 135.57% (P<0.01) in B(a)P alone group. The decrease ranged from 101.31 to 115.68% in both the doses of aloin and hesperidin when compared to B(a)P alone group. The decrease of 76.45% (P<0.001) and 73.83% (P<0.01) in microsomal LPO was found in the forestomach of mice, which were fed only on the higher dose of aloin and hesperidin respectively when compared to control group.

**EFFECT OF PRETREATMENT OF ALOIN AND HESPERIDIN ON BENZO(A)PYRENE MEDIATED FORESTOMACH LEVELS OF GSH AND GSH-METABOLIZING AND ANTIOXIDANT ENZYMES**

Effect of pretreatment of mice with aloin and hesperidin on benzo(a)pyrene mediated forestomach levels of glutathione (GSH),
glutathione metabolizing and antioxidant enzymes. Benz(a)pyrene (alone)-treatment diminished the levels of GSH, glutathione S-transferase, glutathione reduction, and catalase to GPx 59.37% (P<0.01), catalase 60.07% (P<0.05), GST 52.17% (P<0.001), GR 60.23% (P<0.05) and GSH 45.6% (P<0.05), of their corresponding saline-treated controls values as shown in Fig. 2,3 and 4. Treatment of mice with hesperidin and aloin prior to the treatment with benzo(a)pyrene resulted in the recovery of reduced levels of GSH, GSH metabolising enzymes and antioxidant enzymes. The recovery ranged from 65.32% to 80.24% for lower dose of aloin and hesperidin while at higher dose level, the recovery ranged from 72.54% to 84.5% for aloin and hesperidin of their corresponding saline-treated controls and all the values were statistically significant.

HISTOPATHOLOGICAL OBSERVATIONS

Figure 5, 6, 7, 8, 9 and 10 shows the antipromotion studies of forestomach and fig. 11, 12, 13, 14, 15 and 16 shows the antiinitiation study of forestomach tumorigenesis.

In the benzo(a)pyrene alone group the lesions of papillomatosis and squamous cell carcinoma were encountered in the group. Papillomatosis: The wall of forestomach was extensively thickened due to proliferation of stratified squamous cells lining the mucosa which were formed finger like projections protruding into the lumen. Moderate to extensive keratinisation of superficial stratified squamous cells appeared as pink colour a cellular mass covering the mucosa. The finger like projections composed of multiple cells transforming into keratinised mass. The central area of papillomatous growth had elements of connective tissue. At places, transverse section of papilla or villus surrounded by keratinised layer and the lumina contained cellular debris. Squamous cell carcinoma: the stratified squamous epithelium were pleomorphic characterized by presence of cells in various shapes and sizes,. The most of the nucleus were vesicular with presence of prominent nucleolus. Abnormal mitotic figures were also seen. The proliferated squamous cells formed a clusters, where in the centre had a homogenous pink coloured keratinised mass appeared as epithelial pearl
or cell nest, a characteristic feature of squamous cell carcinoma. Palillomatosis is a benign tumor appeared to common in all groups. However, squamous cell carcinoma is maliganant metastatizing tumor also called as cancer observed only in B(a)P alone group only. Other groups prior or post treatment revealed only papillomatous changes, no marked significant changes were noticed in other groups.

STATISTICAL ANALYSIS

The level of significance between different groups is based on Dunnett's t-test followed by the analysis of variance test.

DISCUSSION

The forestomach tumor model has a positive attribute of the inhibitor or inducer coming in direct contact with the target organ thus minimizing the possibility of metabolic alteration. In the present study, we evaluated the effects of oral supplementation of aloin and hesperidin on B(a)P-induced forestomach carcinogenesis and oxidative stress. Many chemical carcinogens, including B(a)P, undergo metabolic conversion to ultimate carcinogens or inactive metabolites and the covalent binding of B(a)P to DNA is considered a critical step in the process of carcinogenesis (Harris 1985, Swenberg et al. 1985). We found a decrease in the number and incidence of forestomach tumors when aloin and hesperidin was given orally along with carcinogen (B(a)P) treatment. Besides this, the number and incidence of tumors were much lower in hesperidin treated animals as compared to aloin treated animals.

Apart from these agents, other compounds are also shown to be chemopreventive against B(a)P induced carcinogenesis in mice. Zhu et al. (2003) evaluated the inhibitory role of β-carotene in the chemoprevention of gastric and other gastrointestinal cancers. Chen et al. (2003) studied the chemopreventive effect of linoleic acid on mouse forestomach neoplasia induced by BP. Singh et al. (1998) showed the inhibitory effect of curcumin on BP-induced forestomach cancer in mice.
In order to define the action of aloin and hesperidin at different stages of forestomach carcinogenesis, we also studied the anti-tumor initiating efficacy of aloin and hesperidin. We found that pretreatment of aloin and hesperidin oral one month prior to B(a)P treatment resulted in a decrease in the number and incidence of forestomach tumors. As observed earlier, the number and incidence of tumors were much lower in hesperidin treated animals as compared to aloin treated animals.

We also investigated the effect of oral supplementation of aloin and hesperidin on BP mediated oxidative stress. BP treatment resulted in the depletion of tissue GSH, GSH metabolising and antioxidant enzymes which were significantly elevated by the pretreatment of aloin and hesperidin in the forestomach. Increase in GSH following aloin and hesperidin treatment may be due to an increase in the reduction, of oxidized glutathione to GSH as a result of increased, GR activity (Sun 1990). Glutathione-S-transferase is one of the major enzymes systems responsible for detoxification of carcinogens. Thus, GST inducers in some experimental settings may be considered potential inhibitors of carcinogenesis. In this study, we also showed a significant induction of GST activity in the forestomach. Therefore, aloin and hesperidin may enhance elimination of B(a)P metabolites by inducing detoxifying enzymes.

It is well established that B(a)P requires metabolic activation mediated by cytochrome P450 (CYP) dependent monooxygenases, for the generation of its ultimate carcinogen, (+)-anti-7, 8 dihydroxy-9,10-oxy-7, 8, 9, 10- tetrahydrobenzo(a)pyrene [(+)- anti-BaPDE]. Covalent interaction of (+)-anti-BaPDE with nucleophilic sites in DNA is a critical event in BaP induced tumorigenesis. Several different mechanisms exist that can convert (+)-anti-BaPDE to less harmful species and thus protect DNA. These mechanisms include spontaneous hydrolysis to tetrols and ketodiols , non enzymatic as well as enzymatic metabolism to triols and triol epoxide hydration by epoxide hydrolase anf glutathione(GSH) and glutathione-S-transferaseSince BaPDE is a poor substrate for epoxide hydrolase, the most important mechanism of BaPDE inactivation seems to be its conjugation with GSH. (Gozukara et al 1981)
The effects of aloin and hesperidin feeding on forestomach GSH levels and GST activities, which play an important role in cellular detoxification of (+)-anti-BaPDE. Aloin and hesperidin are used in our study to assess the efficacy against B(a)P mediated tumor initiation and promotion in mice forestomach.

The results of this study suggest aloin and hesperidin to be an effective inhibitor of B(a)P induced increase in forestomach carcinogenesis. These compounds resulted in the reversal of depleted levels of glutathione and its metabolising enzymes induced by B(a)P. Aloin and hesperidin also suppressed the malignant conversion of papillomas to carcinomas.

The study shows that these compounds have 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 show the efficacy to scavenge singlet oxygen and thus inhibit the initiation of lipid peroxidation.

Subapriya et al. evaluated the effects of neem leaf extract on N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced gastric carcinogenesis in Wistar rats. Administration of neem leaf extract significantly reduced the incidence of stomach tumors, modulated lipid peroxidation and enhanced antioxidant status in the stomach (Subapriya et al. 2003). Thus it is possible that an increase in activities of these enzymes with simultaneous depletion in the level of LPO may reduce the accumulation of oxidants in the forestomach, thus reducing oxidative stress, which may ultimately, result in lesser oxidative damage to DNA and crucial biomolecules (Meneghini 1997). This study suggests that aloe aloin and flavonoid hesperidin inhibit the tumor promotion induced by B(a)P in mouse forestomach carcinogenesis. Thus, these compounds are potent antitumor promoter in mice forestomach and acts by inhibiting the oxidative stress.

Thus, it can be hypothesize that aloin and hesperidin may inhibit B(a)P-induced forestomach tumorigenesis in mice either by inhibiting the activation of B(a)P or/and by enhancing the detoxification of (+)-anti-BaPDE in the target organ forestomach. The results of the present study
suggest that hesperidin and aloin may inhibit B(a)P induced forestomach tumorigenesis in mice by reducing the activation of B(a)P as well by increasing the detoxification of (+)-anti-BaPDE in the forestomach. In conclusion, the data herein reported show that supplementation of aloin and hesperidin can have a strong cancer chemopreventive role in the forestomach of mice.
Effect of oral feeding of Aloin Hesperidin on B(a)P-induced forestomach tumors

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Number of mice</th>
<th>Tumors/mouse</th>
<th>% inhibition</th>
<th>% tumor incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B(a)P*</td>
<td>30</td>
<td>3.25±0.65*</td>
<td>0</td>
<td>100</td>
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<tr>
<td>A1D+B(a)P</td>
<td>30</td>
<td>1.95±0.24*</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>A2D+B(a)P</td>
<td>30</td>
<td>1.85±0.18**</td>
<td>43</td>
<td>50</td>
</tr>
<tr>
<td>H1D+B(a)P</td>
<td>30</td>
<td>1.79±0.60***</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td>H2D+B(a)P</td>
<td>30</td>
<td>1.39±0.37**</td>
<td>57</td>
<td>45</td>
</tr>
</tbody>
</table>

Table-1: Anti-promotion

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Number of mice</th>
<th>Tumors/mouse</th>
<th>% inhibition</th>
<th>% tumor incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B(a)P*</td>
<td>30</td>
<td>4.8±0.20**</td>
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<td>100</td>
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<tr>
<td>A1D+B(a)P</td>
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<td>3.02±0.30**</td>
<td>37</td>
<td>63</td>
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<tr>
<td>A2D+B(a)P</td>
<td>30</td>
<td>2.88±0.36*</td>
<td>40</td>
<td>55</td>
</tr>
<tr>
<td>H1D+B(a)P</td>
<td>30</td>
<td>2.4±0.24*</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>H2D+B(a)P</td>
<td>30</td>
<td>2.16±0.29***</td>
<td>55</td>
<td>50</td>
</tr>
</tbody>
</table>

*B(a)P was administered in corn oil twice weekly for six weeks at a dose level of 1mg/200μl/mouse. Each value represents the mean ± S. E. Of thirty 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.

B(a)P- Benzo[a]pyrene
Fig. 1: Effect of Aloin and Hesperidin on benzo(a)pyrene induced xanthine oxidase and lipid peroxidase activity on mice forestomach.

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.
B(a)P- Benzo(a)pyrene
Fig. 2: Effect of Aloin and Hesperidin on benzo(a)pyrene induced glutathione peroxidase and catalase activity on mice forestomach.

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
B(a)P- Benzo(a)pyrene
Fig. 3: Effect of Aloin and Hesperidin on benzo(a)pyrene induced glutathione-S-transferase and glutathione reductase activity on mice forestomach.

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 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.

B(a)P- Benzo(a)pyrene
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 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.
B(a)P- Benzo(a)pyrene
Histopathology of anti-promotion studies of mice forestomach

Fig. 5: (10 X 7) Control Group (Normal forestomach)

Fig. 6: (10 X 7) Benzo(a)pyrene alone Group (Squamous cell carcinoma)
Histopathology of anti-promotion studies of mice forestomach

Fig. 7: (10 X 7) Hesperidin Dose1 + B(a)P (Fundic area showing naked villi)

Fig. 8: (10 X 7) Hesperidin Dose2 + B(a)P (Papillomatous growth.)
Histopathology of anti-promotion studies of mice forestomach

Fig. 9: (10 X 7) Aloin Dose1 + B(a)P (Mild papillomatous changes)

Fig. 10: (10 X 7) Aloin Dose2 + B(a)P (Mild papillomatous & normal area.)
Histopathology of anti-initiation studies of mice forestomach

Fig. 11: (40 X 7) Control Group (Normal forestomach)

Fig. 12: (40 X 7) Benzo(a)pyrene alone Group (Squamous cell carcinoma showing epithelial pearl.)
Histopathology of anti-initiation studies of mice forestomach

Fig. 13: (40 X 7) Hesperidin Dose1 + B(a)P (Chief cells in fundic region)

Fig. 14: (40 X 7) Hesperidin Dose2 + B(a)P (Mild papillomatous area normal area.)
Fig. 15: (40 X 7) Aloin Dose 1 + B(a)P (Mild papillomatous changes.)

Fig. 16: (40 X 7) Aloin Dose 2 + B(a)P (Mild papillomatous area & metaplastic changes)