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Chemopreventive Action of Liv.52 on DMBA-induced Papillomagenesis in Skin of Mice

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SUMMARY

DMBA (195 nmol/50µl of acetone/animal) was applied topically over the dorsal skin of the mice and tumors were promoted by repeated applications of croton oil (1% in acetone, three times per week) after two weeks of DMBA application. Skin papillomas appeared in 100% animals in control as well as in groups treated orally with Liv.52 at post-initiational stages and continuously at peri-initiational and post-initiational stages of papillomagenesis. When Liv.52 was given orally at the peri-initiational stage of papillomagenesis, the percentage of mice bearing tumors was 75% and the tumor mean per mouse was reduced to 4.0 ± 1.63 as compared to 7.5 ± 3.54 in the control group after 15 weeks of observation. The tumor mean per mouse was observed to be 4.75 ± 0.55 and 2.5 ± 0.57 in the groups treated orally with Liv.52 at the post-initiational stages and continuously at peri-initiational and post-initiational stages of papillomagenesis respectively. Similarly, the cumulative number of papillomas after 15 weeks was 30 in the control group, which was reduced to 10 in the animals treated with Liv.52 continuously at peri-initiational and post-initiational stages. The cumulative number of papillomas was also reduced to 16 and 19 in animals treated with Liv.52 at peri-initiational and post-initiational stages, respectively.

Liv.52 (The Himalaya Drug Co. Private Ltd. India) is an indigenous preparation containing (%); *Capparis spinosa* (24), *Cichorium intybus* (24), *Solanum nigrum* (12), *Cassia occidentalis* (6), *Terminalia arjuna* (12), *Achillea millefolium* (6), *Tamarix gallica* (6) and Mandur bhasma (10). It is a powerful hepatic stimulant and increases the functional efficiency of liver considerably[1]. It has some protective action[2] against hepatotoxic substances like CCl_4. It is useful in infantile cirrhosis[3], stimulates appetite and promotes a feeling of physical and mental well-being[4]. Liv.52 does not show any side effects and is recommeded as a safe, supportive therapy in chronic, resistant dermatoses without known specific aetiology[5]. Protective effect of Liv.52 has been observed in mice against radiation sickness and dermatitis[6]. Significant enhancement in –SH levels in animals treated with Liv.52 as compared to control animals has been observed[7]. All the above mentioned observations led to the use of Liv.52 as a possible chemopreventive agent in DMBA-induced papillomagenesis in the skin of male Swiss albino mice.

MATERIALS AND METHODS

Animals — Random-bred, 7-8 weeks old male Swiss albino mice (36) were obtained from Animal Facility, AIIMS, New Delhi. The animals were acclimatized in the lab for 2 weeks. The animals were provided with standard mice feed (Hindustan Lever Ltd., India.) and tap water *ad libitum*. The dorsal skin of the animals in the interscapular area was shaven three days before the commencement of the experiment and only those animals in the resting phase of hair-cycle were chosen for the study.

Chemicals — DMBA and croton oil were procured from Sigma Chemicals Co., USA. Liv.52 (obtained from the Himalaya Drug Co., India) was given orally to the animals. DMBA was dissolved in acetone at a concentration of 195 nmol/50 μl. Croton oil was diluted in acetone to give 1% dilution.

The animals were assorted into the following control and experimental groups.

Group I (n=4): A single dose of 195 nmol of DMBA in 50 μl of acetone was applied topically over the shaven area of the skin of the mice. Two weeks later croton oil (100 μl of 1% croton oil in acetone) was applied thrice a week until the end of the experiment (15 weeks).

Group II (n=4): The animals of this group received an oral treatment of Liv.52 (0.1 ml/animal/day) at the peri-initiational stage of papillomagenesis (5 days before and 5 days after the application of DMBA). Croton oil was given as in Group I.

Group III (n=4): All the animals received an oral treatment of Liv.52 (0.1 ml/animal/day) starting from the time of croton oil treatment till the end of 15 weeks of experiment (i.e. at the post-initiational stage). DMBA was given as in group I.

Group IV (n=4): All the animals of this group were treated with Liv.52 (0.1 ml/animal/day) throughout the experimental period i.e., both at the initiation and promotion stage. Croton oil was given as in group I. The experiment was carried out for 15 weeks.

Group V (n=4): The animals of this group received only croton oil treatment which was given as in group I.

Group VI (n=4): Mice of this group received an oral treatment of Liv.52 (0.1 ml/animal/day) throughout the experimental period and croton oil was applied as in group I. However, these animals were not treated with DMBA.

Group VII (n=4): These animals received DMBA treatment as in group I but they did not receive either Liv.52 or croton oil treatment.

Group VIII (n=4): In this group, the animals received an oral treatment of Liv.52 throughout the experimental period (0.1 ml/animal/day) and DMBA as in group I, but were not treated subsequently with croton oil.

Group IX (n=4): Animals of this group were only given an oral treatment of Liv.52 (0.1 ml/animal/day) for 15 weeks.

During the 15 weeks of experiment, the mice were weighed weekly and also at the time of autopsy. They were carefully examined once a week for the presence of skin papillomas and the number of papillomas on each affected mouse was recorded. Skin papillomas were defined as lesions with a diameter greater than 1 mm that were present for atleast two consecutive observations.

RESULTS AND DISCUSSION

The results are presented in Figs 1-3 and Table 1.

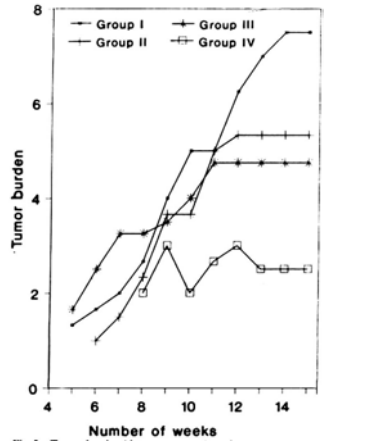

Figure 1: Cumulative number of papillomas in control and experimental groups recorded during the observation period. Group I: Initiator + promoter. Group II: Initiator + promoter + modifier (Liv.52 given at peri-initiational phase of papillomagenesis). Group III: Initiator + promoter + modifier (Liv.52 treatment given at the promotional stage). Group IV: Initiator + promoter + modifier (Liv.52 treatment given both at peri- as well as post-initiational phases).

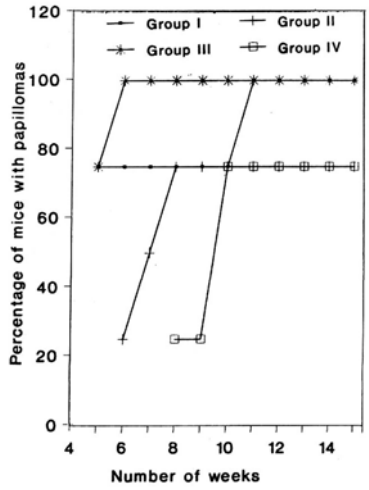

Figure 2: Tumor burden (the average number of tumors per tumor bearing mouse) documented in control and experimental animals. Details of groups are same as in Fig. 1.

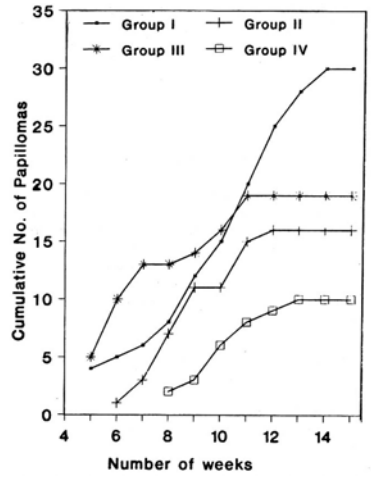

Figure 3: Percentage of mice with papillomas in control and experimental animals. Details of groups are same as in Fig. 1.

Table 1: Tumor response 15 weeks after DMBA and croton oil treatment in Swiss albino mice with or without a treatment of Liv.52.

Groups	No. of mice		% of animals bearing tumors	Tumor mean/mouse ± SE	Av. Wt. Of tumors (mg)	Total no. of papillomas
	Initial	Effective				
I	4	4	100	7.50 ± 3.54	221.75	30
II	4	4	75*	4.00 ± 1.63	165.25	16
III	4	4	100	4.75 ± 0.55	91.25	19
IV	4	4	100	2.50 ± 0.57	22.50	10

*p<0.001 using Chi-square test.

Single topical application of DMBA followed 2 weeks later, by repeated applications of croton oil, in control group (Gr I) resulted in skin papillomas in all (100%) animals and the cumulative number of papillomas induced during the observation period was 30. The mean number of tumor per tumor bearing mouse was 7.5 ± 3.54 and the average tumor weight 221.75 mg. Animals of groups II which received Liv.52 treatment at the peri-initiational phase of tumorigenesis showed only 75% tumor per effective mouse was reduced to 2.5 ± 0.57 and the average tumor weight was observed to be 165.25 mg. All animals in the group III (which were given Liv.52 treatment at the post-initiational stage of tumorigenesis) showed induction of tumors (i.e. 100%) and the cumulative number of tumors was observed to be 19. The mean number of tumor per effective mouse was observed to be 4.75 ± 0.55 and the average tumor weight was 91.25 mg. Mice of group IV (given a continuous treatment of Liv.52 at peri- as well as at the post-initiational phases), which although had a 100% tumor incidence showed a reduction in the cumulative number of papillomas (10) and mean number of tumor per effective mouse (2.5 ± 0.57). The average tumor weight was 22.5 mg

(Table 1). Animals in the rest of the groups did not show any papilloma occurrence during the 15 weeks of observation period.

When given continuously i.e. both at the peri- as well as post-initiational stages, Liv.52 not only lowers the carcinogenic ability of DMBA but also modulates the effects of promoter, i.e. croton oil, therefore, the effect of this treatment is all the more enhanced and the protective effect of Liv.52 is reflected in the decreased values of tumor burden and also the cumulative number of papillomas as compared to the control groups of animals.

Microsomal enzyme studies on Liv.52 treated animals has shown that there is a reduction in malondialdehyde (MDA) formation and Cyt. B5 activity, there is no effect on aryl hydrocarbon hydroxylase (AHH), DT-diaphorase (DTD) and Cyt. P450. However, there was significant enhancement in the GSH levels in animals treated with Liv.52[7]. Liv.52 has also been shown to normalize radiation-induced alterations in GSH levels[7].

Non-protein thiols are known to offer protection by scavenging free radicals. Hence, from a mechanistic point of view, it is possible that Liv.52 may be increasing the detoxification of the carcinogen in the skin by enhancing significantly the GSH levels. Further, it is also possible that the reactive oxygen intermediates generated by the phorbol ester present in croton oil may be scavenged by the SH groups possibly elevated by Liv.52 in the skin of mice.

Thus the present study suggests the potential antipromoting and antitumor activities of Liv.52.

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