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Comparative Study on the Inhibitory Effects of Topical Application and Feeding of Different Egyptian Garlic Extracts on Chemically Induced Skin Tumors

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Abstract: The effects of topical application and feeding of different garlic extracts on 7,12 dimethylbenz(a) anthracene (DMBA)-induced skin tumors in BALB/c mice were investigated. Topical application of ajoene or garlic oil highly prevented the appearance of skin tumors in experimental animals. Carcinomas had never developed , while papillomas had developed in 20% and 16.7% of animals treated with garlic oil and ajoene respectively, in comparison to 6.7% carcinomas and 93.3% papillomas incidence in carcinogenic control mice. Moreover, a significant decrease was observed in number of tumors per mouse and multiplicity. The time till the appearance of first tumor was extended. In contrary to the topical application, garlic extracts (ajoene, garlic oil and fresh minced garlic) had not exerted a significant inhibitory effect against skin tumorigenesis when they were supplemented in diet.

Key words: Garlic, skin tumor, carcinomas, papillomas

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Introduction

Many Scientists focused light on the hazardous effects of various types of cancer treatments, including chemotherapy, radiotherapy and surgical operations. Chemotherapeutic drugs have been found to be far from providing adequate safety in cancer patients (Magrath, 1988). Also, some anticancer drugs had induced neoplastic tumors in some experimental animals El-Mofty *et al.*, 1997). Radiotherapy using various types of ionizing radiation to destroy the malignant tumor cells, usually, results in severe damage of normal cells and organs which are exposed to radiation (Bacq and Alexander, 1961). So, many investigators are searching for new groups of anti-cancer agents to avoid such side effects of chemotherapeutic drugs and radiation. Epidemiological as well as experimental studies have strongly suggested that identification and exploitation of dietary anticarcinogens substantially contribute to the prevention of cancer in humans. Garlic (*Allium sativum*) is a power ful natural medicine hat has been recognized since ancient times (Block, 1985). Antiseptic and antithrombotic activities have been well documented and reduction of serum cholesterol and retarding hyperglycemia attributed to garlic have also been reported (Ernst *et al.*, 1985). In addition raw garlic is a potent antibiotic, especially active against fungal infections, with antibacterial and antiviral reflects as well Hughes and Lawson,1991). Recently, tumor-inhibitory effects of garlic were demonstrated in various experimental systems. An aqueous extract of garlic was shown to inhibit growth of Morris hepatomas (Criss *et al.*, 1982) and the carcinogenesis induced by 3- methylcholanthrene in the terine cervix of virgin albino mice (Hussain *et al.*, 1990). Consumption of both raw garlic and cooked garlic was found to inhibit stomach cancer (Buiatti *et al.*, 1989). Organosulfur compounds of garlic as diallyl sulfide, diallyl disulfide and mercaptan were found to inhibit forestomach carcinogenesis of the female A/J mice (Wattenberg *et al.*, 1989). Wargovich (1988) reported that diallyl sulfide was introduced as suppressing agent in dimethylhydrazine induced colon cancer and nitrosomethyl benzylamine-induced oesophageal cancer development in rats. The phorbol-ester promoted skin tumor formation was demonstrated to be inhibited by topical application of garlic oil (Belman, 1983). Also, El-Mofty *et al.* (1995) demonstrated the inhibition of DMBA-induced skin tumors in BALB/c mice by ajo ene, a natural product from garlic. Futhermore, there is a report concerning the preventive effect of garlic on human cancer; shandong province, China, Gangshan country, where residents consume an average of 20 gm of garlic daily, had the lowest gastric cancer death rate (3.45/100,000) and by contrast, Quixia country, where little garlic is eaten, had the highest (40/100,000) (Horwitz, 1981). Therefore, garlic provides promising agent (s) with the potential to protect against cancer, and thus extensive studies in this field seem to be warranted for public health. An important difficulty in investigating the relation between the use of garlic and cancer development in humans is the determination of actual intake of possible preventive compounds from garlic or garlic supplements. The activity of garlic and garlic preparations is described to compounds containing allyl groups bonded to sulfur (Sumiyoshi & Wargovich, 1990). Recent research on quantification of organosulfur compounds in fresh garlic an d commercially available garlic products revealed considerable variatio n in the results. Accordingly, the main goal of this study was to investigate the inhibitory effects of topical application and feeding of the Egyptian garlic *Allium sativum* and some of its commercial products on skin tumorigenesis induced in female BALB/c mice by DMBA and croton oil.

Materials and Methods

Experimental animals: Young female BALB/c mice, 60 days old and weighing about 15-17 gm were obtained from Institute of Graduate Studies and Research, Alexandria, Egypt. The animals were fed a basal diet composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture, 5% vitaminized starch and vegetables. Animals were allowed tap water *ad libitum*.

Chemical Carcinogens: The polycyclic aromatic hydrocarbon 7,12 dimethyl-benz(a) anthracene (DMBA) was used as an initiator for skin tumor and croton oil was used as a promote. Both chemicals were purchased from Sigma Chemical Co. St. Louis, Mo. USA.

Preventive compounds: For the prevention of tumors, different forms of garlic have been used; these are: a- Garlic oil inside soft gelatineous capsules with the commercial name garlin. It is produced by El-Kahira pharmaceutical and Chemical Ind. Co., Cairo, Egypt. b- Ajoene was kindly supplied by Dr. K. Plank-Schumacher D-3300 Braunschweig , Germany. c- Fresh minced garlic was finely minced before its use.

Tumor induction: Mouse skin tumors were generated by a two-stage nitiation -promotion treatment regimen. An acetone solution (2 ml) containing either the initiator or promoter was topically applied to the dorsal skin of the animals that had been closely clipped 48h earlier. Mice were treated with a single dose of 0.5 mg of DMBA (initiator) and one week later, the mice were ginven 0.5 mg croton oil (Promoter) three times weekly for 35 weeks.

Experimental design: The animals were stored into control and experimental groups of 30 animals each. Different forms of garlic were either topically applied to the dorsal shaved skin of mice or mixed with the diet.

I-Topical application of garlic: Animals of this experiment were divided into 6 groups. Animals of group 1 received no more treatment other than that described in tumor induction and served as carcinogenic controls. In group 2, garlic oil was topically applied at a dose level of 0.2 ml, 30 min after each promotin g dose. In group 3, ajoene (5 mg in 2 ml acetone) was topically applied also 30 min after each promoting dose. The three other groups (group 4,5 and 6) were painted with acetone, garlic oil or ajoene respectively, three times weekly for 35 weeks and used as controls.

II- Feeding of garlic: Animals were divided into 7 groups of 30 mice each. The first four groups were fed on four different kinds of diets for two weeks prior to DMBA application and till the end of the experiment.
The four kinds of diets were prepared as follows:

Diet 1: This diet represented the normal basal diet previously described and was given to animals of group 1 (carcinogenic control).

Diet 2: This diet consists of basal diet mixed with garlic oil at level of 30 ml oil/kg diet, and was given to animals of group 2.

Diet 3: This diet consists of the basal diet mixed with ajoene at a level of 73.3 mg/kg diet, and was given to animals of group 3.

Diet 4: This diet consists of basal diet mixed with fresh minced garlic at a level of 100g/kg diet, and was given to animals of group 4.

The three other groups (group 5,6 and 7) were fed on diets 2,3 or 4 respectively, without receiving any of the carcinogenic substances and used as controls.

Examination of Animals: The experimental animals were carefully examined and weighted weekly. Skin tumor location, number and development were recorded. Histological examination were performed to ascertain the nature of tumors. The experiment was terminated 35 weeks after the beginning of promotion, at which time the mice were autopsied. The percentage of skin tumor-bearing mice was evaluated by X^2 analysis. Tumors per mouse, multiplicity, tumor latencies and body weight gain were analyzed by Student's t-test.

Results

Topical application studies: Table I and Figs 1 & 2 show the effect of topical application of garlic oil and ajoene on the skin tumorigenesis induced by DMBA and croton oil. A

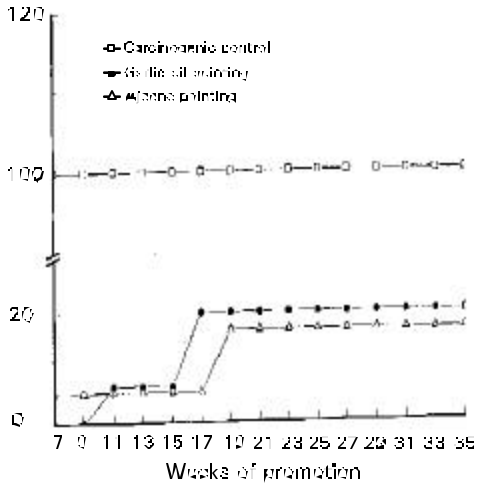

Fig. 1: Effects of painting garlic oil and ajoene on DMBA induced skin tumour in female BALB/c mice. Percentage of mice with tumours.

significant decrease in the percentage of mice bearing papillomas ($p < 0.05$) and carcinomas ($p < 0.001$) was observed in both garlic oil and ajoene treated animals. Carcinomas had never developed, while papillomas were developed in 20% and in 16.7% of garlic oil and ajoene treated animals respectively, in omparison to 6.7% carcinomas and 93.3% papillomas incidence in carcinogenic control mice (Table I and Figs. 3 & 4) In addition to the inhibition of tumor incidence, garlic oil and ajoene had inhibited also tumor occurrence as can be seen by the significant decrease in the number of tumor/mouse ($p < 0.001$) and multiplicity ($P < 0.05$) (Table I). The time till the appearance of the first tumor (latency) was extended significantly ($p < 0.05$) in garlic oil and ajoene treated animals. The first tumor appeared at week 7 in

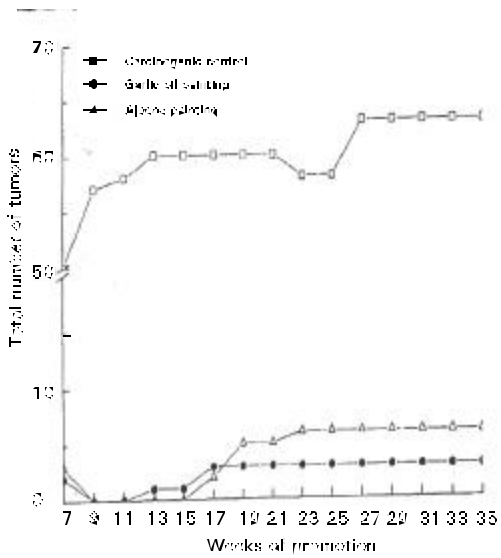

Fig. A induced skin tumours in female BALB/c mice. Total number of tumours

carcinogen control group, while the appearance of the first tumor was postponed to the 13th week and 16th week of tumor promotion in the animals treated with garlic oil and ajoene respectively. None of the mice in the other control groups (groups 4,5 and 6) that survived developed any skin tumors.

Feeding Studies: Table 2 and Fig. 5 & 6 show the effect of feeding garlic oil, ajoene and fresh minced garlic on skin tumorigenesis induced by painting DMBA on the skin of BALB/c mice. Feeding the diet containing garlic oil, ajoene or fresh minced garlic led to insignificant changes in the incidence of skin tumors, number of tumor/mouse, tumor multiplicity and the latency, compared with the carcinogenic controls. The percentage of mice bearing tumors was 100% in carcinogenic control group in comparison to 78.9% in garlic oil treated group, 82.4% and 88.9% in ajoene and fresh minced garlic fed groups respectively (Table 2). 14 mice out of 15 in carcinogenic control group were found to have skin papillomas and only one had squamous cell carcinoma. On the other hand., all the tumors observed in garlic treated groups (groups 2, 3 and 4) were papillomas.

Neither tumor nor pathological changes were observed in the skin of control mice in groups 5,6 and 7. There was no appreciable fall in the body weight (data not given) in experimental animals of both topical application and feeding studies.

Discussion

The present study demonstrates the preventive action of topical application of garlic oil and ajoene on DMBA-initiated croton oil promoted skin carcinogenesis . The two garlic forms resulted in significant reduction in the tumour incidence, tumor yield, multiplicity and significant increase in the latency. Ajoene showed greater inhibitory effect than garlic oil and the

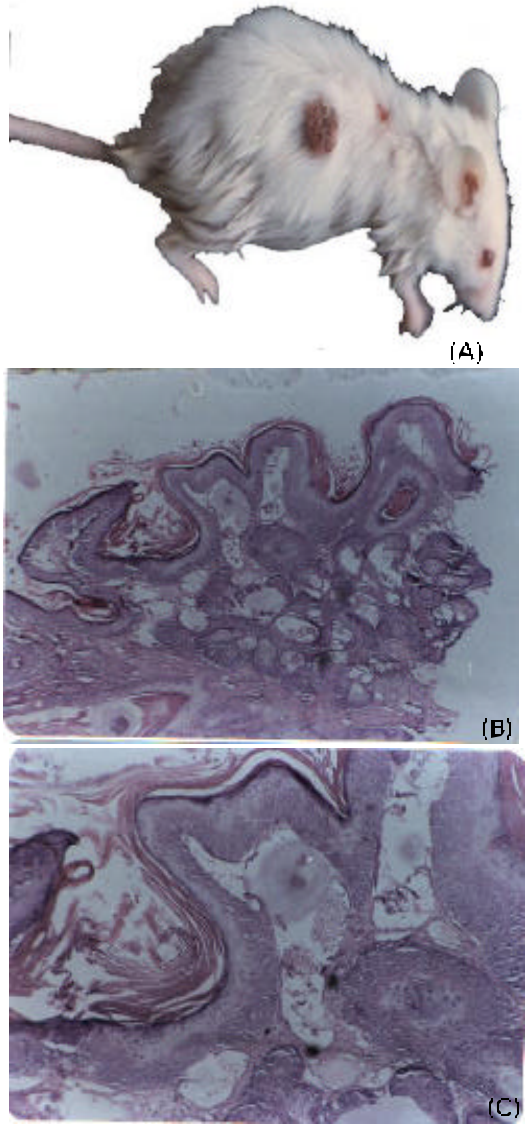

Fig. 3: A) An experimental mouse with papillomas in the chest and back (X1.5).
B) A case of papilloma showing multiple papillae with fibrovascular cores (X 100)
C) A higher view of the previous case showing acenthosis and hyperkeratosis of the covering epithelium (X 300)

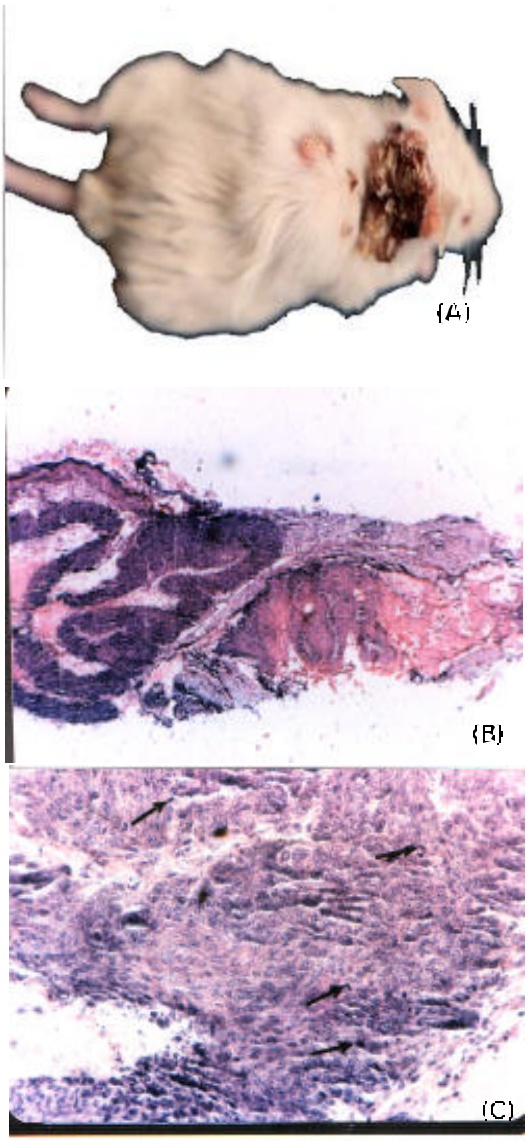

Fig. 4: A) An experimental mouse with advanced skin tumor diagnosed as squamous cell carcinoma (X 1.5)
B) A case of squamous cell carcinoma showing stratified squamous epithelium from which masses of tumor tissue are seen to arise (X 100).
C) A high power veiw of the previous case showing plemorphic malignant squamous cells with increase mitotic activity (arrows) (X 500).

latent period of tumor appearance was increased in animals treated with ajoene compared with those given garlic oil. This suggests the absence of some ajoene components in garlic oil. Inhibition of chemical carcinogenesis has been achieved by a number of naturally occurring as well as ynthetic substances (Birt, 1986). The fact that garlic oil has the preventive action on DMBA-initiated croton oil-promoted skin carcinogenesis was first reported by patniak *et al.* (1980). Belman (1983) showed inhibion of DMBA-induced phorbolmyristate-acetate-induced skin papillomas by garlic oil. Furthermore, sadhana *et al.* (1988) demonstrated inhibition of bP-induced and croton-oil promoted skin carcinogenesis by garlic oil. The preventive

Table 1: Effect of topical application of garlic oil and ajoene on skin tumorigenesis in female BALB/c mice painted with DMBA and croton oil

Group	Treatment	No of Survivors	No of mice bearing Tumors		Total no of tumors	No of tumours[a] per mouse	Multiplicity[a,b]	Latency [a,c]
			Papillomas	Carcinomas				
1	DMBA/croton oil (Carcinogenic control)	15	14(93.3%)	1(6.7%)	63	4.2±0.17	4.2±0.17	7.13±0.13
2	DMBA/Croton oil+garlic oil	15	3(20%)**	0(0%)	3	0.2±0.11**	1±0.28	14.33±1.67*
3	DMBA/Croton oil+ajoene	18	3(16.7)**	0(0%)	6	0.33±0.21*	2.0±0.58	17.5±0.87
4	Acetone	21	0(0%)	0(0%)	0	0	0	0
5	Garlic oil	19	0(0%)	0(0%)	0	0	0	0
6	Ajone	21	0(0%)	0(0%)	0	0	0	0

* Significant at $P<0.05$ ** Singnificant at $P<0.001$ a= Number expressed as mean ±SE

b= Multiplicity is defined as number of tumors per tumor bearing mouse.

C= Latency is defined as the time, in weeks, from begining of promotion to the appreance of the first tumor in mice

Table 2: Effect of feeding of garlic oil, ajoene and fresh minced garlic on skin tumorigenesis in female BALB/c mice painted with DMBA and croton oil.

Group	Treatment	No of Survivors	No of mice bearing Tumors		Total no of tumors	No of tumours[a] per mouse	Multiplicity[a,b]	Latency [a,c]
			Papillomas	Carcinomas				
1	DMBA/croton oil +bascal diet (diet 1)	15	14(93.3%)	1(6.7%)	63	4.2±0.17	4.2±0.17	7.13±0.13
2	DMBA/Croton oil+garlic oil (diet 2) 9.33±0.13		19	15(78.9%)	0(0%)	49	2.58±0.22	3.24±0.12
3	DMBA/Croton oil+ajoene (diet 3)	17	14(82.4%)	0(0%)	43	2.53±0.19	3.07±0.17	9.07±0.19
4	DMBA/conton oil+ fresh minced garlic (diet 4)	18	16(88.9%)	0(0%)	57	3.17±0.16	3.7±0.16	10.25±0.25
5	Garlic oil (diet 2)	18	0(0%)	0(0%)	0	0	0	0
6	Ajone (diet 3)	17	0(0%)	0(0%)	0	0	0	0
7	Fresh minced garlic (diet 4)	19	0(0%)	0(0%)	0	0	0	0

a= Number expressed as mean ±SE

b= Multiplicity is defined as number of tumors per tumor bearing mouse.

C= Latency is defined as the time, in weeks, from begining of promotion to the appreance of the first tumor in mice

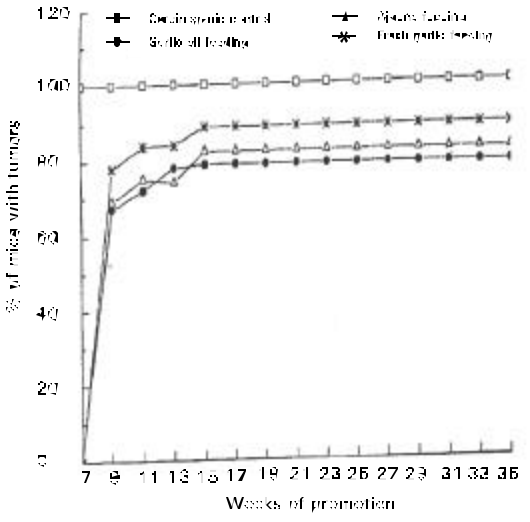

Fig. 5: Effect of feeding garlic oil, ajoene, and fresh minced garlic on DMBA-induced skin tumors in female BALB/c mice. Percentage of mice with tumors

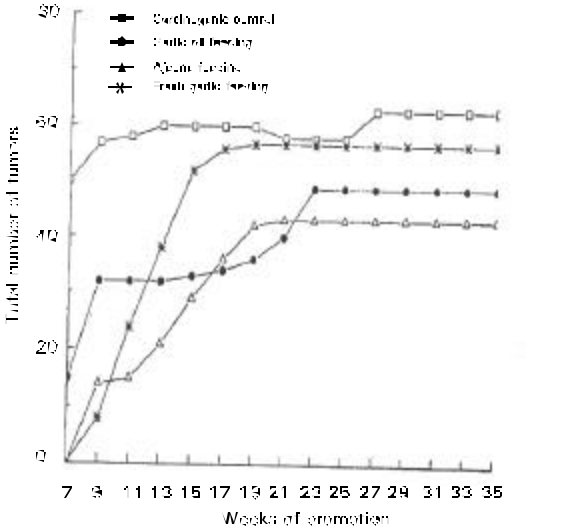

Fig. 6: Effect of feeding garlic oil, ajoene, and fresh minced garlic on DMBA-induced skin tumors in female BALB/c mice. Total number of tumors

effect of ajoene was demonstrated byBelman *et al.* (1987) who studied the inhibition of skin tumor promotion in female HA/ICR mice using ajoene. Scharfenberg *et al.* (1990) reported that ajoene exerted a stronger effect on the viability d tumorigenic cells than of non tumorigenic cells.

Garlic oil and garlic extracts was found to reduce the percentage of mice with skin tumors as well as the number of tumor per tumor-bearing mouse (Rao *et al.*, 1990). Also El-Mofty *et al.* (1999) demonstrated the inhibitory effect of fresh minced garlic and ajoene on Aflatoxin-B1 induced carcinogenesis in toads. Sparnins *et al.*, (1988) suggested that allyl containing compounds and fresh garlic extract inhibited the formation of malignant tumors induced by various initiators. The chemical composition of garlic has been defined (Block, 1985). Garlic was considered an excellent source of organo-sulfur compounds, and it has been proposed that these compounds are probably responsible for some of the cancer-preventing activity of garlic. On contrary to their topical application, the garlic extracts (garlic oil, ajoene and fresh min ced garlic) has not exerted a significant inhibitory effect against skin tumorigenesis when they were supplemented in diets.

The beneficial effect of garlic compounds on skin tumor inhibition was explained by Watson (1983) who stated that garlic may enhance the production of some lymphokines, such as the tumor necrosis factor, which may account for the impressive destruction of tumor in laboratory animals. From the biochemical point of view, the inhibitory effect of garlic compounds on skin tumors can be explained by stimulation and elevation of the natural, antioxidative, protective functions within the organism that are directly coupled to the redox system of glutathione (GSH) and oxidized glutathione (GSSG). As support of this proposal, garlic oils were all found to enhance the activity of glutathione peroxidase in epidermis cells that had been pre treated with the tumor promoter (Perchellet *et al.*,1990). Additionally, garlic oil was found to block ornithine decarboxylase (ODC) activity in epidermis cells. ODC-induction, caused by phorbol ester and other promoters, is prevented by the enhanced activity of GSH peroxidase (Rozhin *et al.*, 1984). Another hypothesis to explain the inhibitory effect of garlic is stimulation of glutathione that reacts with electrophilic sites produced on the carcinogen molecule by the cytochrome P 450 hydroxylase, this by blocking their nucleophilic attack on DNA (Zeilkoff *et al.*, 1986). Results of the present work, showed that different garlic compounds caused great inhibitory effect on skin tumorigenesis when they were topically applied, but mixing them with diets, did not show any significant effect. This could be explained if we consider the mode of application of painting compounds. When they were applied topically, their constituents were in close contact with the transformed cells or to the cells found in the area of carcinogen/promoter application. Therefore, they can modulate the promotion phase of carcinogenesis by exerting a local effect directly on the epidermal cells or on the metabolic pathway of the promoter itself, especially, if we took into consideration that garlic oils were immediately applied after the application of the promoter.

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