Cancer Therapy Vol 5, 301-308, 2007

Green tea treatment of ultraviolet-B (UVB) skin carcinogenesis in mice

Research Article

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Key words: Green tea treatment, UVB skin carcinogenesis

Abbreviations: ultraviolet-B, (UVB)

Received: 28 November 2006; Revised: 7 April 2007
Accepted: 10 September 2007; electronically published: September 2007

Summary

The experiment investigated the role of green tea treatment of UVB skin photocarcinogenesis with or without the co-carcinogen arsenite. 260 mice were divided into six groups each of 36. Group (1) was control. Group (2) received sodium arsenite in water. Group (3) was UVB radiated. Group (4) was arsenite treated and UVB radiated. Group (5) was UVB radiated and treated with green tea. Group (6) was radiated with arsenite and green tea treated. Specimens from skin, lymph nodes, lung, liver, kidneys, and spleen were taken at 9 months from group (I) & (2) and monthly after four months exposure in the other groups. Paraffin sections were stained by H&E. UVB radiation induced: dysplastic changes in epidermis, preneoplastic hyperplasia of rete pegs, hyperplasia of hair follicles, trichofolliculoma, trichofolliculocarcinoma, squamous cell carcinoma, basal cell carcinoma and fibropapilloma. Green tea treatment prevented the induction of rete pegs preneoplastic hyperplasia, hair follicle hyperplasia, trichofolliculocarcinoma, squamous cell carcinoma, fibropapilloma, rhabdomyosarcoma and mixed tumors. Green tea reduced the incidence and delayed the appearance of dysplastic changes and trichofolliculoma. Arsenite has no co-carcinogenic effect. Green tea has no significant effect in the arsenite UVB group.

I. Introduction

Ultra violet radiation is one of the main etiologic agents of skin cancer. Epithelial papillomas, fibropapillomas, keratoacanthomas, benign pigmented macules, squamous cell carcinoma, fibrosarcomas and cutaneous melanomas were experimentally produced (Kligman and Kligman, 1981 and Strickland et al, 2000). Squamous cell carcinoma and eye cancers occurred naturally (Nikula et al, 1992; de Gruijl and Van der Leun, 2000).

Arsenite acts as a co-carcinogen with UVB radiation by inhibiting DNA repair and/or enhancing positive growth signaling (Rossman et al, 2001).

Green tea polyphenols (GTPs) as antioxidants prevented the formation of skin tumors and protected against UVB photocarcinogenesis by preventing lipid peroxidation of epidermal microsomes (Wang et al, 1994; Huang et al, 1997; Record and Drosti, 1998; Zhao et al, 1999; Katyar et al, 2000; Lu et al, 2001; Conney et al, 2002).

The work aim to investigate the role of green tea in prevention of photocarcinogenesis and the role of sodium arsenite as cocarcinogen.

II. Materials and methods

A. Materials

1. Apparatus

Philips TL 20/12 sunlamp (TTS product and service, Germany).

2. Chemicals

Sodium arsenite: (British Drug Houses LTD, England), Green tea: (China Tea import and export corporation, China).

3. Experimental animals

260 adult albino mice 3 months age and 25-30 gm weight were kept for one week. They received food and water ad liptum. They were shaved one day before the beginning of the experiment.

4. Experimental design

Mice were divided into 6 groups each of 36. Group I was kept as a control. Group 2 received sodium arsenite via drinking water at concentration of 10 mg per liter. Group 3 was radiated 4 hrs. daily, 3 times per week (each next day) at a dose of 200 J/m^2 for 9 months . The distance from the lamp and the backs of mice was 20 cm. Group 4 was given sodium arsenite and radiated. Group 5 was given green tea in a concentration of 6 mg per ml as sole source of drinking fluid and radiated. Group 6 was given green tea, arsenite and radiated.

B. Methods

1. Sampling

Tissue specimens from different areas of the skin and from lung, liver, spleen as well as subscapular and prefemoral LNs were taken. In groups 3,4,5,6, monthly specimens were taken after 4 months exposure. The rest of the surviving mice as well as those of group 1 and 2 were slaughtered at the end of the experiment (9 months).

2. Histopathological examination

Specimens were embedded in paraffin. 7 micron sections were stained by H&E (Bancroft and Stevens, 1982).

3. Statistical analysis

The significance differences between groups were tested by Mann – Whitney test, in 1947.

III. Results

UVB radiation induced all the stages of neoplasia from dysplastic changes, preneoplasia, benign and malignant tumors **(Table 1).** The significance differences between groups are listed in **Table 2**.

UVB radiation induced epidermal dysplastic changes in form of focal acanthosis **(Figure 1)**, loss of polarity **(Figure 2)**, variation in size and shape of nuclei, nuclear aggregation, presence of prominent nucleoli **(Figure 3),** mitosis, cytoplasmic basophilia and loss of keratohyaline granules. The percent of dysplasia was 38.1% after 4 months exposure in UVB group. Green tea treatment reduced the percent to 7.1 % and delayed the appearance to 9 months. In the UVB arsenite group the percent was 35.7 at 4 months. Green tea reduced it to 25% and delayed its appearance to 7 months.

Table 1. Tumours initiation in the experimental groups.

	1-Control group			2-Arsenite group			3-UVB group			4-UVB and green tea group			5-UVB and arsenite group			6-UVB, arsenite and green tea group		
	No	%	***	No	%	***	No	%	*	No	%	*	No	%	*	No	%	*
Number of mice	36	-	'	36	-	'	36	-	-	36	-	-	36	-	-	36	-	-
Exposed cases **	-	-	-	-	-	-	21	-	-	13	-	-	14	-	-	16	7 9 -	-
Dysplastic changes	-	-	-	-	-	-	8	38.1	4	1	7.7	9	5	35.7	4	4	25	
Preneoplastic hyperplasia of rete pegs	-	-	-	-	-	-	2	9.5	9	-	-	-	1	7.1	5	1	6.3	
Hyperplasia of hair follicle	-	-	-	-	-	-	3	14.3	6	-	-	-	1	7.1	4	-	-	-
Trichofolliculoma	-	-	-	-	-	-	6	28.6	7	2	15.4	4	5	35.7	6	4	25	9
Trichofolliculocarcinoma	-	-	-	-	-		1	4.8	4	-	-	-	-	-	-	-	-	-
Sebaceous adenocarcinoma	-	-	-	-	-	-	-	-	-	1	7.7	4	-	-	-	1	6.3	6
Squamous cell carcinoma	-	-	-	-	-	-	2	9.5	6	-	-	-	-	-	-	-	-	-
Basal cell carcinoma	-	-	-	-	-	-	1	4.8	4	1	7.7	8	-	-	-	1	6.3	6
Rhabdomyosarcoma	-	-	-	-	-	-	1	4.8	4	-	-	-	2	14.3	5	1	6.3	6
Liposarcoma	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	6.3	6
Prolymphocytic lymphosarcoma	-	-	-	-	-	-	-	-	-	-	-	-	1	7.1	6	-	-	-
Mixed tumours	-	-	-	-	-	-	2	9.5	4	-	-	-	-	-	-	1	6.3	9
Types of mixed tumors:							Case number 1: Trichofolliculocarcinoma, Rhabdomyosarcoma, Basal cell carcinoma Case number 2: Trichofolliculoma Fibropapilloma 9									One case Trichofolliculoma, Sebaceous adenocarcinoma Liposarcoma, Rhabdomyoscarcoma.		
Fibropapilloma							1	4.8	9	-	-	-	-	-	-	-	-	-
No. of non affected cases							3	14.3	-	8	61.5	-	1	7.1	-	8	50	

* Time of death or slaughter in months.
** The exposed animals are those receiving UVB for the whole time of the experiment.
***The animals were slaughtered at the end of 9 months.

Table 2. Statistical differences between groups using Mann-Whitney test.

	Mann-Whitney U	Exact sig. [2*(1-tailed sig.)]
Between UVB group and UVB and green tea group	37.000	0.014*
Between UVB group and UVB and arsenite group	56.000	0.15
Between UVB group and UVB, arsenite and green tea group	59.500	0.204
Between UVB and green tea group and UVB, arsenite and green tea group	57.500	0.169
Between UVB and arsenite group and UVB, arsenite and green tea group	78.500	0.762

* Significantly different at P≤ 0.05

Preneoplastic hyperplasia of rete pegs was 9.5% after 9 months in UVB group (**Figure 4**). It was prevented by green tea treatment. The percent was 7.1 at 5 months in UVB arsenite group and 6.3 % at 9 months when green tea treated. Hyperplasia of hair follicles in both treated UVB and UVB arsenite groups (14.3% at 6 months and 7.1% at 4 months) was prevented by green tea.

The hair follicle was the most sensitive structure to be affected. Trichofolliculoma (**Figures 5, 6**) was induced in 28.6% after 7 months in UVB and 35.7% after 6 months in UVB arsenite group. Green tea reduced the percent to 15.4 and 25% and delayed the appearance to 4 months and 9 months respectively. Malignancy as trichofolliculocarcinoma (**Figures 7, 8**) was observed in 4.8% after 4 months in UVB group and prevented by green tea treatment. It was not observed in the UVB arsenite group or treated one.

UVB single or with arsenite did not induce sebaceous adeno-carcinoma, while a single case (7.1% and 6.3% after 4 and 6 months respectively was observed in each treated counterpart groups (**Figures 9, 10**).

Squamous cell carcinoma with lung metastasis appeared for the first time at 6 months with incidence of 9.5% in UVB group. It was abscent in the other 3 groups. Green tea prevented squamous cell carcinoma.

Sporadic case of basal cell carcinoma (**Figures 11, 12**) was observed in 3 groups of UVB (4.8% after 4 months), UVB and green tea (7.7% after 8 months) and in the UVB, arsenite and green tea (6.3% after 6 months). There was no tumor induction in the UVB and arsenite group. The effect of treatment and cocarcinogen can not be concluded.

The incidence of rhabdomyosarcoma (**Figures 13, 14**) was 4.8 % after 4 months and prevented by green tea treatment. Arsenite cocarcinogen effect raised the incidence to 14.3% at 5 months that was decreased to 6.3% by green tea treatment and delayed to 6 months.

Liposarcoma (**Figure 15**) was observed only as sporadic case (6.3 % after 6 months) in the UVB, arsenite and green tea group. No conclusion about treatment and co-carcinogen could be taken. Prolymphocytic lymphosarcoma was observed as sporadic case (7.1% after 6 months) in the UVB and arsenite group.

Two cases of mixed tumors were observed in the UVB group with incidence of 9.5 %. The type of one case was tricofolliculocarcinoma with rhabdomyosarcoma and basal cell carcinoma 4 months post exposure. The other case was trichofolliculoma, with fibropappilloma at 9 months. One case of mixed tumors with incidence of 6.3% after 9 months was observed in UVB arsenite and green tea group. The tumor was a mixture of trichofolliculoma, sebaceous adenocarcinoma, basal cell carcinoma, liposarcoma, and rhabdomyocarsoma. Arsenite has no cocarcinogen effect in general.

Fibropapilloma was observed in one case 4.8% after 9 months in UVB group and prevented by green tea. It was not observed in UVB arsenite group or treated one.

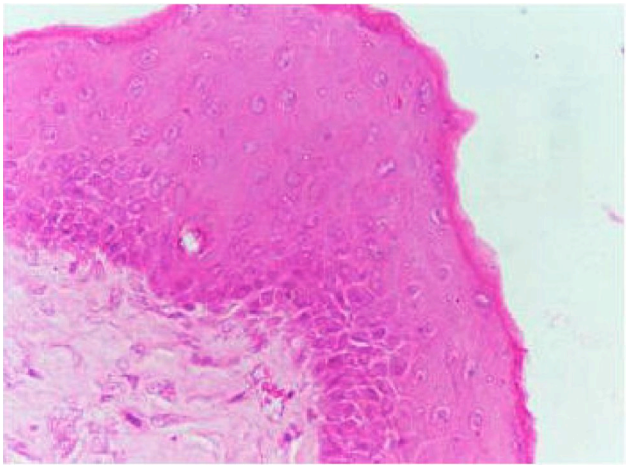

Figure 1. Hyperplastic changes in epidermis. Focal acanthosis. Group 6 (H&Ex25).

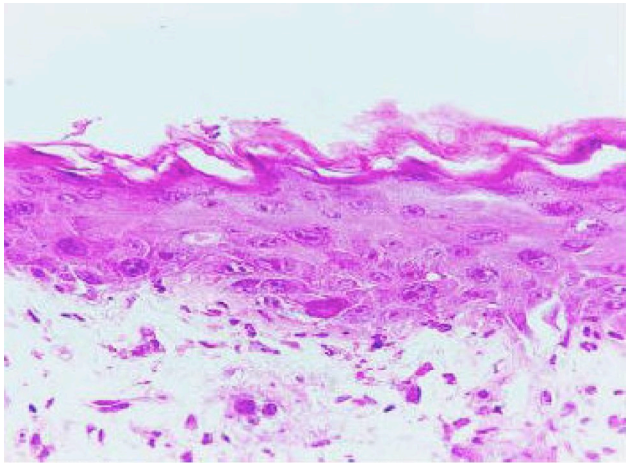

Figure 2. Dysplastic changes in epidermis. Loss of polarity. Group 6 (H&Ex40).

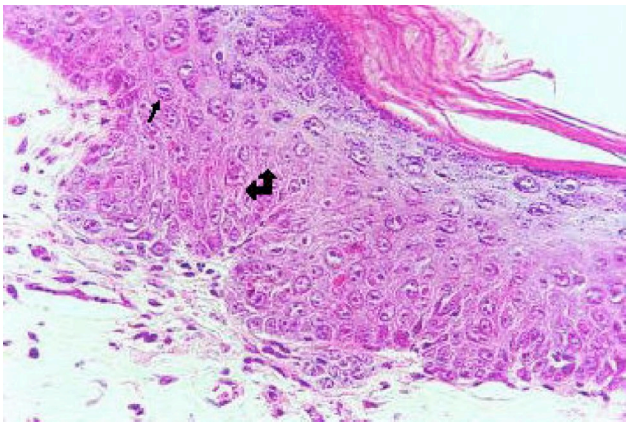

Figure 3. Dysplastic changes in epidermis. Nuclear aggregation and variation in size and shape of nuclei (bifurcated arrow). Presence of prominent nucleoli (arrow). Group 6 (H&Ex40).

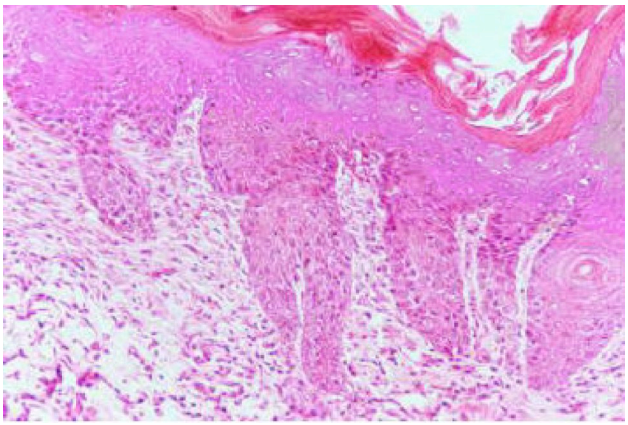

Figure 4. Preneoplastic hyperplasia of rete pegs to form hair follicles without differentiation of trichohyaline forming cells and sebaceous glands. Group 6 (H&Ex25).

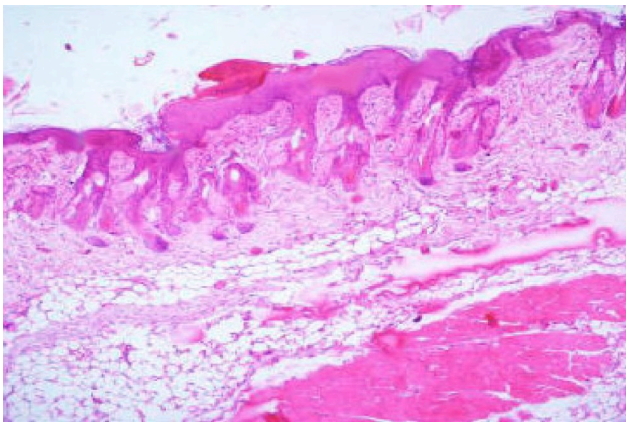

Figure 5. Trichofolliculoma. Expansive growth. Compound follicles (arrow). Moderate number of hair follicle with variation in size and distribution. Group 6. (H&Ex10).

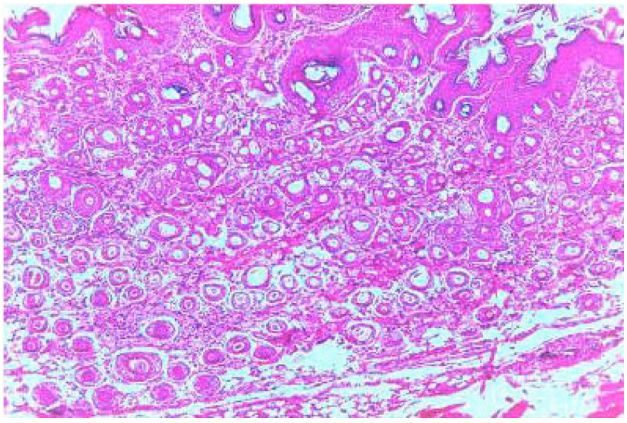

Figure 6. Trichofolliculoma. Expansive growth and high number of hair follicles. Group 6. (H&Ex10).

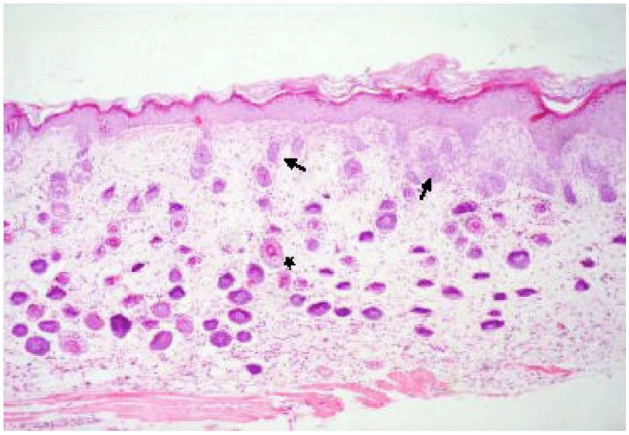

Figure 7. Trichofolliculocarcinoma. Diffuse infiltration of dermis by undifferentiated (arrows) and partial differentiated hair follicles (star). Group 3. (H&Ex4).

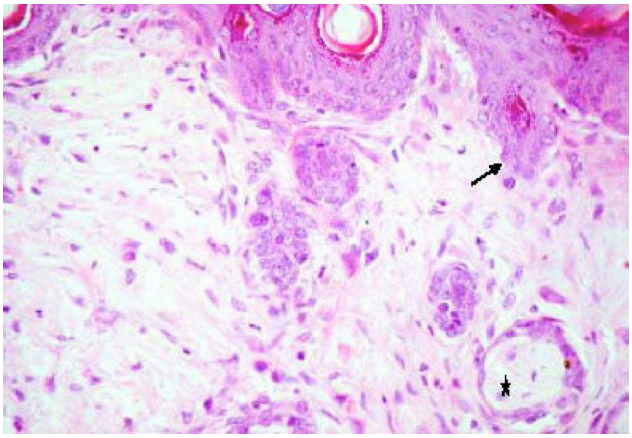

Figure 8. Trichofolliculocarcinoma. Tumor cells budding from germinativum. Partial differentiation to trichohyaline (arrow). Partial differentiation to sebaceous gland (star). Group 3 (H&Ex40).

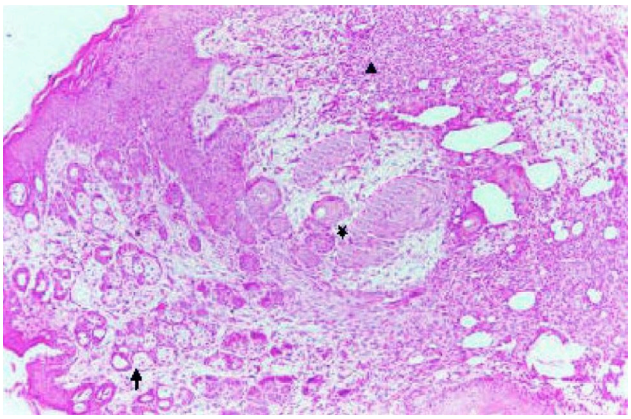

Figure 9. Sebaceous adenocarcinoma. Infiltrative growth of undifferentiated (arrows) and partial differentiated (star) sebaceous adeni. Diffuse infiltration of undifferentiated sebaceous cells (arrow head). Group 6. (H&Ex10).

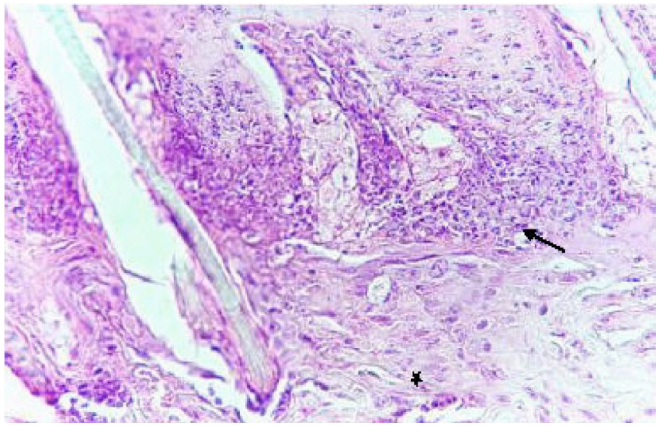

Figure 10. Sebaceous adenocarcinoma. Diffuse infiltration of undifferentiated (arrows) and partial differentiated (star) sebaceous cells. Group 6. (H&Ex40).

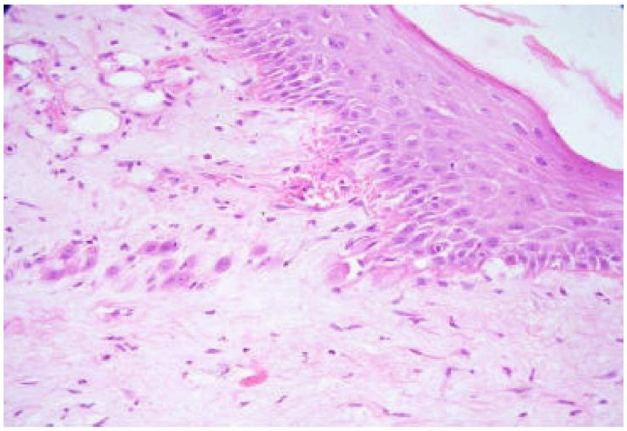

Figure 11. Basal cell carcinoma. Basal cells infiltrate dermis. Group 6 (H&Ex40).

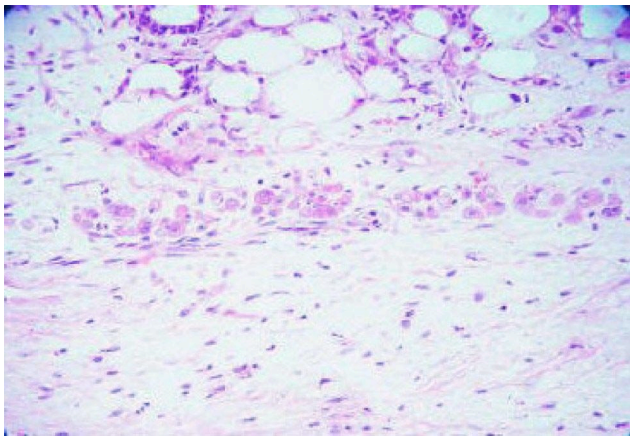

Figure 12. Basal cell carcinoma. Basal cells infiltrate hypodermis. Group 6 (H&Ex40).

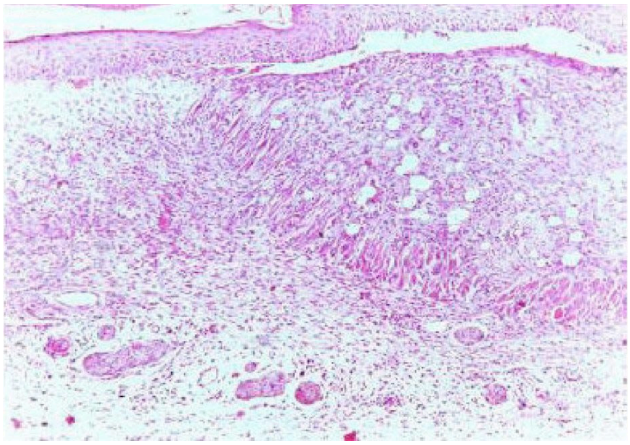

Figure 13. Rhabdomyosarcoma. Rhabdomyoblast cell bundles invading upward to the dermis. Group 6 (H&Ex10)

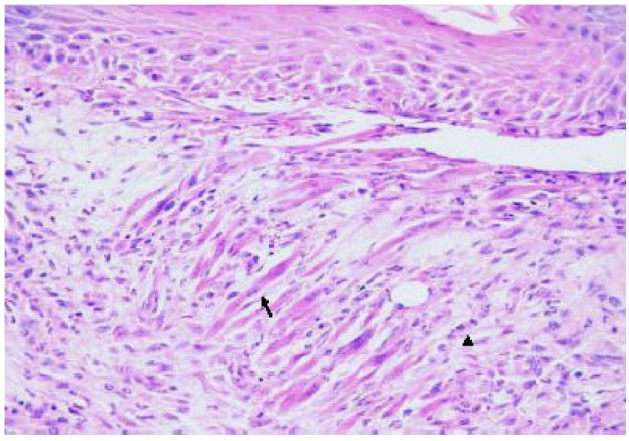

Figure 14. Rhabdomyosarcoma. Spindle cells of different sizes; many are abnormally elongated (arrow). Basophilic cytoplasm. Hyperchromatic nuclei. Some cells contain more than one nuclei (arrow head). Group 6 (H&Ex40).

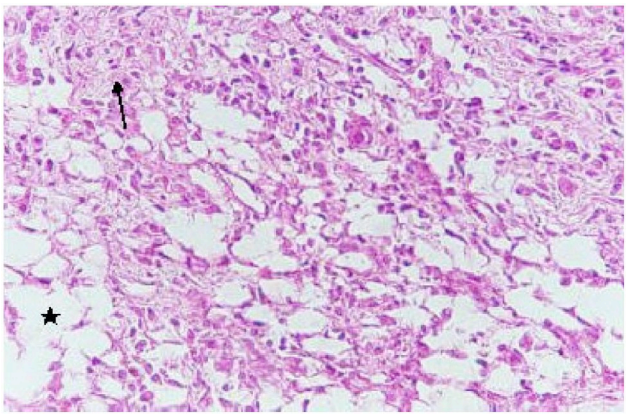

Figure 15. Liposarcoma. Lipocytes (star). Lipoblast cells (arrow). Group 6 (H&Ex40).

IV. Discussion

UVB radiation induced all the stages of neoplasia from dysplastic, preneoplastic hyperplasia, benign and malignant tumors. Stenback in 1978, Canfield and colleagues in 1986 and Pentland and colleagues in 1999 recorded UVB skin dysplasia and Rossman and colleagues in 2001 hyperplasia of hair follicles.

All components of epidermis, dermis and adenexia responded to UVB radiation. The appearance of mixed tumors confirmed this fact. The response was directly malignant more than benign. Malignant transformation included epidermis to squamous cell carcinoma and basal cell carcinoma; sebaceous glands to sebaceous adenocarcinoma; adipose cells to liposarcoma; muscles to rhabdomyosarcoma and subcutaneous lymph nodes to prolymphocytic lymphosarcoma.

Squamous cell carcinoma was the most commonly skin tumor produced by UVR (Pentland et al, 1999; Rossman et al, 2001). In this experiment arsenite has no co-carcinogen effect. Rossman et al, 2001 stated that arsenite with UVR enhanced the appearance of SCC to be earlier, the size to be larger and the growth to be more invasive. The appearance of basal cell carcinoma ranged from 4 to 8 months. Aszterbaum and colleagues founded in 1999that the incidence increases with chronic exposure to UVR alone.

Records for UVB, induction of sebaceous adenocarcinoma, liposarcoma and rhabdomyosarcoma were not cited in the literature. Pentland and colleagues reported in 1999 prolymphocytic lymphosarcoma.

The hair follicle was the most sensitive structure to be affected by UVB radiation. Trichofolliculoma was observed in 17 cases among all groups. Trichofolliculocarcinoma in one case. UVB induction of these tumors was not found in the literature. Trichofolliculoma is considered more commonly as hamartoma (Walder and Gross, 1992). Fibropapilloma was induced experimentally by UVR by Kligman and Kligman in 1981 and Scott and colleagues in 2001.

Arsenite has no carcinogenic effect in general. It may be true for lymphoid tissue and muscles. Numerous attempts for arsenic co-carcinogenesis have mostly failed (Larc, 1980; Wang and Rossman, 1996; Germolec et al, 1977).

Green tea treatment prevented: preneoplastic hyperplasia of rete pegs, hyperplasia of hair follicles, trichofolliculocarcinoma, fibropapilloma, squamous cell carcinoma, rhabdomyosarcoma, liposarcoma, prolymphocytic lymphosarcoma, and mixed tumors. Green tea treatment reduced the incidence of and delayed the appearance of dysplastic changes and trichofolliculoma.

Green tea inhibited the induction of UVR skin tumors (Wang et al, 1994; Lou et al, 1999; Lu et al, 2001; Conney et al, 2002. The antioxidant capacity of green tea resulted in decreased UVB enhanced lipid peroxidation (Katiyar et al, 1994). UVR lead to release of cytokines inducing immunosuppression which is an important factor for skin tumor induction (Dummer et al, 1995; Kim et al, 1995 and Granstein, 1996). UV induced IL-10 can be blocked by using antioxidants (Katiyar, 1999).

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