CHEMOPREVENTIVE ACTION OF GINSENG ON DMBA-INDUCED PAPILLOMAGENESIS IN THE SKIN OF MICE

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Ginseng is an oriental herbal remedy which has been in use for the last 5000 years. Extensive research has shown that Ginseng contains medicinally useful substances such as Ginsenosides, Minerals - iron, copper, zinc, Mn, Mg, Germanium, phosphorus, sulphur etc. Enzymes, carbohydrates, aminoacids, proteins, peptides, flavonoids, essential oils, fatty acids, phytosterols, crude fibers, adenosine, maltol and phenolic compounds. Ginseng has been shown to possess pharmacological properties including "antifatigue" and "antistress" actions normalizing effects on carbohydrate metabolism, and a stimulating effect on the central nervous system. It has also adaptogenic property.

All these observations led to the use of Ginseng as a possible chemopreventive agent in DMBA (7, 12-Dimethylbenz(a) anthracene) induced papillomagenesis in the skin of male Swiss albino mice.

MATERIALS AND METHODS

Animals :

Random-bred, male Swiss albino mice (7-8 weeks old) were obtained from Animal facility, AIIMS, New Dehli. 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. Ginseng was given intraperitionally to the animals.

DMBA was dissolved in acetone at a concentration of 50 μg/50 μl. Croton oil was diluted in acetone to give 1% dilution.

Experimental Design :

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

Group 1 :

A single dose of 50 μg of DMBA in 50 μl of acetone was applied topically over the shaven areas of the skin of mice. Two weeks later croton oil (100 μl of 1% croton oil in acetone) was applied three times/week until the end of the experiment (15 weeks).

Group 2 :

The animals of this group received Ginseng treatment (7.0 mg/kg body wt./day) 5 days before and 5 days after the application of DMBA. Croton oil was given as in group 1.

Group 3 :

All the animals received Ginseng treatment (7.0 mg/kg body wt./day) starting from the time of croton oil treatment till the end of 15 weeks of experiment. DMBA was given as in group 1.

Group 4 :

All the animals of this group were treated with Ginseng (7.0 mg/kg body wt./day) throughout the experimental periold i.e. before and after DMBA application and also at the promotional stage. Croton oil was given as in group 1. The experiment was carried out for 15 weeks.

Group 5 :

The animals of this group were treated with only croton oil, which was given as in group 1.

Group 6 :

Mice of this group received Ginseng treatment (7.0 mg/kg body wt./day) throughout the experimental period. Croton oil was applied as in group 1. However, these animals were not treated with DMBA.

Group 7 :

These animals received DMBA treatment as in group 1 but they did not receive either Ginseng or croton oil treatment.

Group 8 :

In this group the animals received Ginseng treatment throughout the experimental period (7.0 mg/kg body wt./day) and DMBA as in group 1 but were not treated subsequently with croton oil.

Group 9 :

Animals of this group were only given Ginseng treatment (7.0 ㎎/㎏ body wt./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 at least two consecutive observations.

RESULTS

The results of the present investigations are depicted in Table. 1. Further Fig. 1. depicts the percentage of mice with papillomas in control (Group 1) and experimental groups (2, 3, 4) lots. Fig. 2. depicts the number of tumors per tumor bearing mouse documented during the observation period in control and experimental groups. Fig. 3. depicts the cumulative number of tumors induced during the observation period in control and experimental animals.

Table 1. Chemopreventive effect of Ginseng on DMBA - induced papillomagenesis in the skin of mice

Group	Treatment			Animal No.		Body weight(g) Mean± S.D.		Mice with Papillomas		Papillomas bearing
No.	Modulator	Initiator	Promoter	Initial	Effective	Initial	Final	(%)	mouse	
1	Nil	DMBA	Croton oil	25	23	30.0± 0.2	35.4 ± 2.1	23/23	(100.0)	6.3 ± 2.7
2	Ginseng									
	(a) Peri-initiational phase	DMBA	Croton oil	25	23	28.0± 0.25	36.05± 3.0	13/23	(56.5)	3.1 ± 0.68
	(b) Post-initiational phase	DMBA	Croton oil	25	23	29.0± 0.6	35.4 ± 2.8	18/23	(85.7)	4.56± 0.57
	(c) Peri-post initiational phase	DMBA	Croton oil	25	25	30.6± 1.5	36.5 ± 3.2	8/25	(32.0)	1.8 ± 0.37
3	Ginseng	Nil	Croton oil	25	25	31.3± 0.9	35.9 ± 0.9	0/25	0.00	0.00
4	Ginseng	DMBA	Nil	25	25	28.0± 0.25	31.4 ± 1.2	0/25	0.00	0.00
5	Nil	Nil	Croton oil	25	25	29.5± 1.05	34.3 ± 1.3	0/25	0.00	0.00

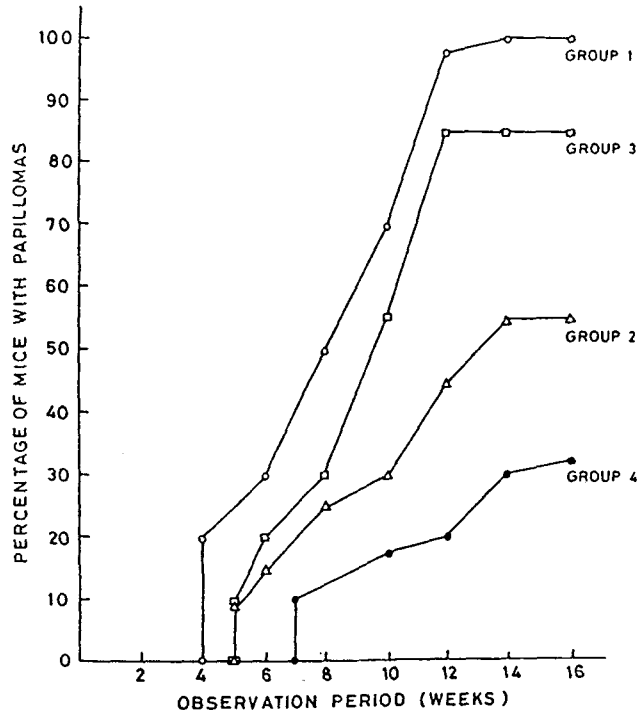

Fig. 1

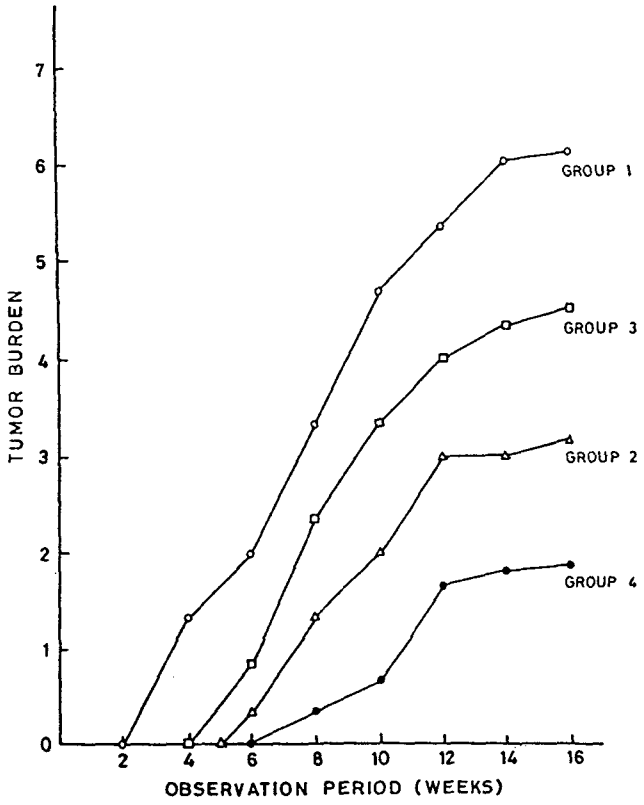

Fig. 2

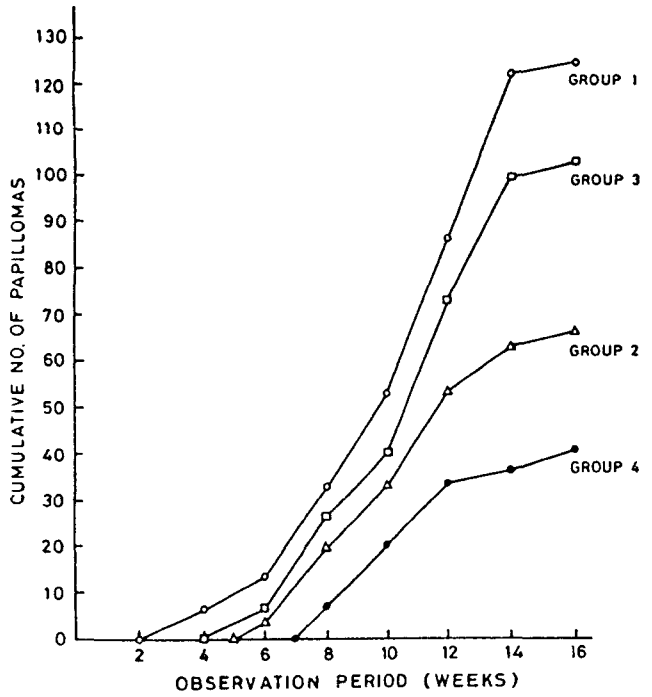

Fig. 3

In the control group (gr. I) in which a single topical application of DMBA was followed, 2 weeks later, by repeated applications of croton oil, skin papillomas appeared in all (100%) animals and the cumulative number of papillomas induced during the observation period was 126. The average number of tumors per tumor bearing mouse was observed to be 6.3± 2.7. Animals of group II which received Ginseng treatment at the peri - initiational phase of tumorgenesis showed only 56.5% tumor incidence and the cumulative number of papillomas was only 65. The mean number of tumors per effective mouse was reduced to 3.1± 0.68. All animals in group III (Which were given Ginseng treatment at the promotional stage of tumorigenesis) showed induction of tumors (i.e. 85.7%) and the cumulative number of tumors was observed to be 102. The average number of tumors per effective mouse was observed to be 4.56± 0.57. Mice of group IV (given a continuous treatment of Ginseng at peri - as well as at the post - initiational phases, showed a 32% tumor incidence, and a reduction in the cumulative number of papillomas (42) and average number of tumors per effective mouse (1.8± 0.37). Animals in the rest of the groups did not show any papilloma occurrence during the 15 weeks of observation period.

DISCUSSION

There are several reports and reviews on the use of naturally occurring as well as synthetic substance as chemopreventive agents in different animal tumor model systems[1~3].

The present investigation demonstrates the chemopreventive potential of Ginseng on DMBA induced skin tumorigenesis in male Swiss albino mice. The finding shows that there is reduction not only in tumor incidence but also in cumulative tumor frequency and tumor burden following exposure of DMBA treated once to Ginseng not only during the peri and post initiation phase but during the peri - initiational phase alone also and to some extent during the post - initiation phase also.

We have demonstrated that Ginseng treated animal show reduction in malondialdehyde formation and cytochrome b5 activity, there is no effect on aryl hydrocarbon hydroxylase, DT - diaphorase and cytochrome P - 450.

However, there was significant enhancement in the GSH levels. In addition this study revealed that the Ginseng could augment the level of soluble SH groups in the liver of mice.

Ginseng has also been reported to have significant amount of phenolic compound and vitamins. This GSH/GST enzyme system and the presence of phenols has been reported to be responsible for the conjugation of xenobiotic with glutathione and thus brings cellular detoxification[4~8].

It is possible that Ginseng by increasing the GSH contents and phenolic substance could potentially decrease the carcinogenic effect of DMBA.

Further work involving *in vivo* as well as *in vitro* studies, is required to assess the magnitude and the mechanism of chemoprevention of carcinogenesis by Ginseng or by its active principles.

REFERENCES

1. Bonne, C.W., Kelloff, G.J. and Malone, W.E.(1990) : Identification of candidate cancer chemopreventive agents and their evaluation in animal models and human clinical trials : A Review Cancer Res., 50, 2 - 9.
2. Wattenberg, L.W. (1985) : Chemoprevention of cancer. Cancer Res., 45, 1 - 8.
3. Williams, G.W. (1984) : Modulation of chemical carcinogenesis by xenobiotics. Fund. Appl. Toxical., 4, 325 - 344.
4. New Mark, H.L.(1987) : Plant phenolics as inhibitors of mutational and precarcinogenic events. Can. J. Physiol. Pharmacol. 65, 461 - 466.
5. Ketterer, B. Protective role of glutathione and glutathione transferases in mutagenesis and carcinogenesis. Mut. Res. 202 : 343 - 361.(1988).
6. Orrenius, S., Moldeus, P. The multiple roles of glutathione in drug metabolism. Trends pharmacol. Sci. 5 : 432 - 435, 1984.
7. Coles, B., Ketterer, B(1990) : The role of glutathione and glutathione S - transferases in chemical carcinogenesis CRC. Int. Rev. Biochem. Mol Biol. 25 : 47 - 70.
8. Meister, A., Anderson, M.E.(1983) Glutathione, Ann. Rev. Biochem. 52 : 711 - 760.