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Research article

Chemoprevention of chemical-induced skin cancer by *Panax ginseng* root extract

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ABSTRACT

Background: Cancer has emerged as a major health problem globally as a consequence to the increased longevity of the population, changing the environment and life style. Chemoprevention is a new and promising strategy for reducing cancer burden. Recently, some natural products have been identified for their chemopreventive activity to reduce the cancer incidence. Ginseng is known for its potential to treat various ailments in human beings. The present study was designed to explore the anticancer and antioxidative potential of *Panax ginseng* against chemical-induced skin carcinogenesis in mammals.

Methods: Skin tumors were induced in Swiss albino mice by a single topical application of 7,12-dimethylbenz(a)anthracene (100 μg/100 μL acetone) and, 2 wks later, promoted by repeated applications of croton oil (thrice in a wk in 1% acetone) till the end of the experiment (i.e., 16 wk). Hydro-alcoholic ginseng root extract at a dose of 25 mg/kg body weight/d was orally administered at the peri-initiation, postinitiation, and peri–post-initiation stages.

Results: Ginseng root extract treatment caused a significant reduction in tumor incidence, cumulative number of tumors, tumor yield, and tumor burden, as compared to the 7,12-dimethylbenz(a)anthracene–croton oil-treated control group. Further, biochemical assays revealed a significant enhancement in the levels of reduced glutathione, superoxide dismutase, catalase, vitamin C, and total proteins but a significant reduction in lipid peroxidation levels in both the liver and skin with ginseng root extract treatment, as compared to carcinogen-treated control group.

Conclusion: These results suggest that *P. ginseng* has the potential to become a pivotal chemopreventive agent that can reduce cancer in mammals.

1. Introduction

Cancer has emerged as a serious global threat; it is the second leading noncommunicable disease following cardiovascular disease in the developed nations and the third fatal disease in developing countries such as India. A great melioration in existing cancer treatment methods is still ineffective to reduce the global burden of this disease. The World Cancer Report (2014), developed by the International Agency for Research on Cancer, predicts an acclivity of new cancer cases from an annual estimation of 14 million in 2012 to 22 million within 2 decades. Over the same period, cancer deaths are expected to increase from 8.2 million to 13 million/yr. Exposure to various chemicals, biological agents, and dietary habits as well as life style contribute to cancer occurrence and in the probability of diagnosis [1]. However, 30–40% of cancer incidences can be prevented by healthy dietary intervention and regular physical activity.

The World Health Organization had launched a campaign against cancer, with a three-fold strategy: prevent all the preventable cancers, cure all that can be cured, and reduce pain and discomfort where cure is not possible [2]. The two most effective ways to bring down the cancer risk are as follows: avoidance of cancer-causing biological, chemical, and physical agents and an increased intake of diet that has a chemopreventive action.

Skin carcinogenesis is growing rapidly in the developed countries due to the depletion of the ozone layer and changes in environmental composition. Due to the high incidence of skin cancer in Australia and European countries, it is regarded as a dreaded

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disease there [3]. The multistage skin carcinogenesis model is a widely acceptable model to test tumor development at initiation and promotion stages, and the preventive approach of drugs can be easily evaluated [4].

Chemoprevention is the administration of natural or synthetic compounds to prevent, slow down, and reverse the occurrence of cancer. Various civilizations across the world still rely on herbal medicines as the first line of treatment. Owing to their safety, low toxicity, antioxidant properties, cost effectiveness, and general acceptance (as dietary supplements, fruits, vegetables, phytochemicals, and minerals), these are being investigated for the prevention of cancer. Extensive research over the past few decades has identified numerous dietary and botanical natural compounds that have anticancerous properties [5–7].

Panax ginseng belongs to the family Araliaceae and is commonly grown in Korea. Ginseng is acclaimed as the magical herb that acts as a restorative and prophylactic agent to treat various maladies such as cancer. The active constituents of *P. ginseng* include ginsenosides, essential oil, peptidoglycans, polysaccharides, nitrogen-containing compounds, fatty acids, and phenolic compounds. Both below- and above-ground parts of this plant have medicinal properties [8,9]. The root extract of ginseng has been empirically used in Korea, Japan, and China for its role as an anticarcinogenic, antidiabetic, antistress, antifatigue, anti-inflammatory, antioxidant, and radioprotective agent [10–13].

Owing to the presence of a wide range of medicinal constituents in *P. ginseng*, the present study was designed to evaluate the antioxidative and antitumorigenic potential of this plant extract in a mammalian model using a two-stage skin carcinogenesis protocol.

2. Materials and methods

2.1. Chemicals

The initiator 7,12-dimethylbenz(a)anthracene (DMBA) and the promoter croton oil were procured from Sigma Chemicals Co. (St Louis, MO, USA). DMBA was dissolved at a concentration of 100 μg/100 μL in acetone. Croton oil was mixed in acetone to give a solution of 1% dilution.

2.2. Animals

The protocol of the experiment was approved by the Institutional Ethical Committee (1678/GO/a/12/CPCSEA), and animal care and handling were done according to the guidelines set by the World Health Organization, Geneva (Switzerland) and the Indian National Science Academy, New Delhi (India). The present study was conducted on Swiss albino mice (7–8-wk old and weighing 24 ± 2 g) selected from a random breed inbreed colony. These animals were housed in polypropylene cages in the animal house under controlled conditions of temperature (25 ± 2°C) and light (14 light:10 dark). The animals were fed a standard mouse feed (procured from Aashirwad Industries, Chandigarh, India) and water was given *ad libitum*.

2.3. Phytochemical profile of ginseng root

Korean Red Ginseng "Insam" was purchased from market. They were 6-yr old fresh ginseng roots (main roots 70% + fine roots 30%) with 14% moisture content. The ginsenoside (Rb1 + Rg1) content was 7.74 mg/g. Moreover, 30 types of ginsenosides and acidic polysaccharides were present (Table 1).

2.4. Preparations of ginseng root extract

Red ginseng root powder was purchased, and its hydroalcoholic extract was prepared by refluxing it with double-distilled water and alcohol (3:1) in a round bottom flask for 36 hours (3 × 12 hrs) at 60°C. The liquid extract was filtered, cooled, and concentrated by evaporating its liquid content using an oven, and collected. The powdered extract, termed ginseng root extract (GRE), was dissolved again in double-distilled water prior to its oral administration in mice.

2.5. Optimum dose selection

Different doses [5 mg/kg/body weight (b.wt)/d, 10 mg/kg/b.wt/d, 25 mg/kg/b.wt/d, 50 mg/kg/b.wt/d, 100 mg/kg/b.wt/d, and 200 mg/kg/b.wt/d] of GRE were given orally for 15 consecutive days, and the morphological and behavioral changes were recorded for 30 d. The biochemical changes in the levels of lipid peroxidation (LPO), glutathione (GSH), and total proteins in liver were estimated after the 16th- and 30th-d autopsy. The dose showing the highest levels of GSH and total proteins, and the lowest levels of LPO was selected to carry out further experimentation.

The required dose for treatment was prepared by dissolving the extract in double-distilled water at a dose of 25 mg/kg/b.wt.

2.6. Experimental design

Animals for this study were divided into the following groups:

Group I: vehicle-treated control group (normal) Mice (n = 10) belonging to this group received a topical application of acetone (100 μL/mouse) on the shaven dorsal skin, and double-distilled water (equivalent to GRE, i.e., 100 μL/mouse/d) by oral gavage for 16 wks.
Group II: GRE-treated control group (negative control) Animals (n = 10) belonging to this group received GRE (25 mg/kg/b.wt/animal/d) by oral gavage throughout the experimental period, i.e., 16 wks, and acted as drug-treated controls.
Group III: carcinogen-treated control group (positive control) Mice (n = 10) belonging to this group were treated with a single topical application of 100 μL DMBA (100 μg/100 μL acetone) over the shaven area of the skin. Two wks later, 100 μL croton oil (1% w/v in acetone) was applied topically three times per wk until the end of the experiment.
Group IV: GRE-treated experimental group (peri-initiation) These animals (n = 10) received the same treatment of carcinogen as Group III, and the GRE was orally administered at a dose of

Table 1
Phytochemical profile in the korean red ginseng root extract

Sample no.	Constituents	Examples		Reference
1.	Total saponins (ginsenosides)	Protopanaxadiol (20S) Protopanaxatriol (20S) Oleanolic acid	Ra1, Ra2, Ra3, Rb1, Rb2, Rb3, R4, Rs1, Rs2, Rs3, Rs4, Rc, Rd Re, Rf, Rg1, R1 Ro	Fuzzati 2004 [45]
2.	Polysaccharide	Panaxane A–U		Kwak et al 2010 [46]
3.	Volatile oils	A-cadinol, α-bisabolol, thujopsene, and n-hexadecanoic acid		Qui et al 2008 [47]
4.	Rb1/Rg1 ratio	1–3		Chen et al 2008 [48]

25 mg/kg/b.wt/animal/d, starting from 7 d prior to the DMBA application to 7 d after the application.

Group V: GRE-treated experimental group (postinitiation) The treatment pattern of DMBA and croton oil was the same as that in Group III. This group ($n = 10$) received GRE (25 mg/kg/b.wt/animal/d) by oral gavage, starting from the time of croton oil application until the end of the experiment.

Group VI: GRE-treated experimental group (peri- and post-initiation) Animals ($n = 10$) of this group were administered GRE (25 mg/kg/b.wt/animal/d) orally, starting at 7 d prior to DMBA application and continuing throughout the experimental duration (i.e., 16 wks). The carcinogen treatment was the same as that in Group III.

2.7. Induction of tumor

For the induction of skin tumors, dorsal hairs between the cervical and caudal portions of the animals of Groups III–VI were removed using a surgical clipper, 2 d prior to the initiation of the experiment, and 100 μL DMBA (100 μg/100 μL acetone) was applied. After 14 d, the tumor initiation by DMBA was promoted by the topical application of 100 μL croton seed oil (1% v/v in acetone), thrice per wk, for the next 14 wks.

During the 16 wks of experimentation, all mice were observed daily and weighed weekly. Tumors appearing on the shaven area of the skin were examined and recorded at weekly intervals in all the above groups. Only those tumors that persisted for ≥ 2 wks, with a diameter of > 2 mm, have been taken into consideration for the final evaluation of the data. Skin tumors, regressed after one observation, were not accounted.

2.8. Morphological study

2.8.1. Cumulative number of tumors

The total number of tumors appeared till the termination of the experiment were estimated.

2.8.2. Tumor incidence

The number of mice carrying at least one tumor was expressed as a percentage incidence.

2.8.3. Tumor yield

The average number of tumors per mouse was calculated.

2.8.4. Tumor burden

The average number of tumors per tumor-bearing mouse was estimated.

2.8.5. Diameter

The diameter of each tumor was measured.

2.8.6. Weight

The weight of each tumor was measured at the termination of experiment.

2.8.7. Body weight

The weight of each mouse was measured weekly.

2.8.8. Average latent period

The time lag between the application of the promoting agent and the appearance of 50% of tumors was determined. The average latent period was calculated by multiplying the number of tumors appearing each wk by the time in wks after the application of the promoting agent, and dividing the sum by the total number of tumors.

$$\text{Average latent period} = \frac{\sum FX}{N}$$

where F is the number of tumors appearing each wk, X is the numbers of wks, and N is the total number of tumors.

2.8.9. Inhibition of tumor multiplicity

$$\frac{\text{Total number of tumors in carcinogen} - \text{treated control} - \text{total number of tumors in GRE treated}}{\text{Total number of tumors in carcinogen} - \text{treated control}} \times 100$$

2.9. Biochemical study

The animals from all the groups were sacrificed by cervical dislocation 16 wks after the commencement of treatment, and their liver and dorsal skin that were affected by tumors were quickly excised and washed thoroughly with chilled 0.9% NaCl (pH 7.4). Both of the tissues (liver and skin) were then weighed and blotted dry. A 10% tissue homogenate was prepared from the part of the tissue sample in 0.15M Tris-KCL (pH 7.4) to estimate the reduced glutathione and LPO levels.

2.9.1. Reduced glutathione

The level of GSH was estimated as total nonprotein sulfhydryl group by the method of Moron et al [14]. Free endogenous-SH was assayed, and the absorbance was recorded at 412 nm using a UV–VIS Systronics spectrophotometer. Reduced GSH was used as a standard. The levels of GSH were expressed as μmol/g of tissue.

2.9.2. Lipid peroxidation

The LPO level was calculated spectrophotometrically by the thiobarbituric acid reactive substances method, as described by Ohkhawa et al [15]. The optical density of LPO was observed at 532 nm, and the content of thiobarbituric acid reactive substances was expressed as nmol/mg of tissue.

2.9.3. Superoxide dismutase

Superoxide dismutase (SOD) level was determined according to the method of Marklund and Marklund [16] by quantification of pyrogallol auto-oxidation inhibition, and the results were expressed as units/mg protein. Auto-oxidation of pyrogallol in Tris-HCL buffer (50mM, pH 7.5) was measured by the increase in absorbance at 420 nm.

2.9.4. Catalase

Catalase activity was measured by the method of Aebi [17]. Phosphate buffer (50mM) was used for homogenate preparation

Table 2
Antitumorigenic potential of ginseng root extract on chemical-induced skin carcinogenesis in mice

Treatment group[1)]	Body weight (g)		No. of tumor	Tumor weight (g)
	Initial	Final		
Normal	25.47 ± 5.15	35.74 ± 6.60	—	—
Negative control	26.94 ± 3.86	34.47 ± 6.29	—	—
Positive control	26.31 ± 3.44	24.19 ± 4.93	57.00 ± 4.58	1.99 ± 0.22
Peri-initiation	26.12 ± 5.56	30.54 ± 6.73*	39.67 ± 4.73***	1.2 ± 0.19***
Postinitiation	25.87 ± 4.20	31.78 ± 3.54**	30.33 ± 4.04***	0.83 ± 0.19***
Peri–post-initiation	27.45 ± 4.11	32.89 ± 5.28**	17.67 ± 2.52***	0.51 ± 0.06***

Data are presented as mean ± SD
Statistical comparison: control versus experimental
*$p < 0.05$
**$p < 0.01$
***$p < 0.001$
SD, standard deviation
[1)] Treatment schedule of the groups is specified in materials and methods

and centrifuged at 4307 *g* for 10 min. The change in absorbance was observed spectrophotometrically at 240 nm. The activity of the enzyme was expressed as U/mg of tissue, where U was μmol of H_2O_2 disappearance/min.

2.9.5. Vitamin C

For this, the skin and liver tissue were weighed, homogenized in acetate buffer (20 mg/mL), extracted with cold 4% trichloroacetic acid, centrifuged, and filtered. Ascorbic acid was determined by the method of Roe and Kuether [18].

2.9.6. Total proteins

Total protein content of the skin and liver was estimated by the method of Lowry et al [19] by preparing homogenate in distilled water, and absorbance was recorded at 670 nm. Protein concentration was measured from a standard curve of bovine serum albumin and the level was expressed as mg/g.

2.10. Histopathological study

On the completion of the 16th wk, the skin and tumor tissue samples were collected for histopathological examination. Tissues were treated in a series of alcohol grades, and blocks were prepared by embedding in paraffin wax. Permanent slides were developed by cutting 5-μm-thick sections, stained with hematoxylin and eosin, and observed under a light microscope.

2.11. Statistical study

Data from different experimental groups were analyzed and expressed as mean ± standard deviation. The morphological data were evaluated using Student *t* test by Bourke et al [20]. The significant levels in biochemical parameters were statistically computed using an analysis of variance (Stat Plus 2009, AnalystSoft Inc company for Windows.), followed by Bonferroni test for differences in means. A *p* value < 0.05 was considered significant for all experiments.

3. Results

3.1. Morphological study

As shown in Table 2, treatment with the GRE significantly affected the various stages of skin carcinogenesis in mice. The body weight was found to be gradually increased during experimentation in Group I, but it was found to decrease in the carcinogen-treated control animals.

Animals of Group III exhibited 100% tumor incidence after the treatment with DMBA/croton oil alone, while the animals of Groups I and II did not show any tumor appearance (Fig. 1).

The cumulative number of tumors in the carcinogen-treated control group was noted as 57 ± 4.58, which were significantly ($p < 0.001$) reduced to 39.67 ± 4.73 in the peri-initiated group, 30.33 ± 4.04 in the postinitiated group, and 17.67 ± 2.52 in the peri–post-initiated group after GRE administration. The tumor yield exhibited a significant ($p < 0.001$) decline, i.e., 3.97 ± 0.47, 3.03 ± 0.40, and 1.77 ± 0.25 in the GRE-treated Groups IV–VI, respectively, when compared with Group III. The tumor burden was noted as 5.7 ± 0.46 in the carcinogen-treated control group, and it was also significantly ($p < 0.001$) decreased to 4.99 ± 0.71, 4.60 ± 0.98, and 3.67 ± 1.15 after the administration of GRE in the experimental groups. Groups IV–VI showed an appreciable elevation in the average latent period (11.13 ± 0.34, 12.02 ± 0.14, and 12.86 ± 0.28, respectively) in comparison to the control group (9.86 ± 0.08; Table 3; Fig. 1).

3.2. Biochemical study

LPO level was found to be significantly ($p < 0.001$) elevated in the liver and skin of the animals in the carcinogen-treated control group, when compared with the normal group. The administration of the GRE in the experimental groups showed a significant inhibition of LPO in Group IV ($p < 0.05$), Group V ($p < 0.001$), and Group VI ($p < 0.001$) in both the tissues (Tables 4 and 5).

The GSH level exhibited a significant reduction in the positive control group when compared with the normal group, and the treatment with GRE significantly restored the GSH activity in the skin and liver of the animals in the peri-initiated ($p < 0.01$; $p < 0.05$), postinitiated ($p < 0.001$, $p < 0.01$), and peri–post-initiated ($p < 0.001$; $p < 0.001$) groups (Tables 4 and 5).

In comparison with normal mice, the levels of antioxidant enzymes (SOD and catalase) in the liver and skin were observed to

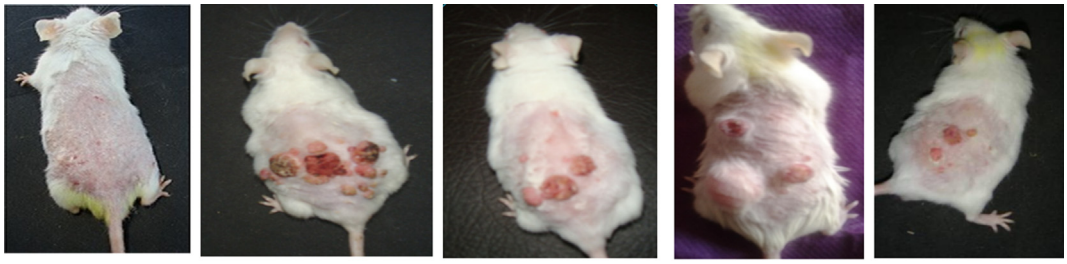

Fig. 1. Morphological variations in tumor appearance during chemical-induced skin carcinogenesis with or without GRE administration in different groups. GRE, ginseng root extract.

Table 3
Chemopreventive potential of GRE on chemical-induced skin carcinogenesis in mice

Treatment group[1)]	Tumor yield	Tumor burden	Average latent period	Inhibition of tumor multiplicity (%)	Tumor incidence (%)
Normal	—	—	—	—	—
Negative control	—	—	—	—	—
Positive control	5.7 ± 0.46	5.7 ± 0.46	9.86 ± 0.08	—	100
Peri-initiation	3.97 ± 0.47***	4.99 ± 0.71*	11.13 ± 0.34***	30.40	80
Postinitiation	3.03 ± 0.40***	4.60 ± 0.99*	12.02 ± 0.14***	46.79	66.67
Peri−post-initiation	1.77 ± 0.25***	3.67 ± 1.15*	12.86 ± 0.28***	69	50

Data are presented as mean ± SD
Statistical comparison: control versus experimental
*$p < 0.05$
**$p < 0.01$
***$p < 0.001$
GRE, ginseng root extract; SD, standard deviation
1) Treatment schedule of the groups is specified in materials and methods

Table 4
Variations in different biochemical parameters in liver during chemical-induced skin carcinogenesis with or without GRE administration

Treatment group	LPO[1)]	GSH[2)]	SOD[3)]	CAT[4)]	Vit. C[5)]	Total protein[6)]
Normal	2.57 ± 1.09	7.99 ± 0.67	69.02 ± 5.57	46.38 ± 6.22	2.48 ± 0.38	111.21 ± 15.38
Negative control	1.91 ± 1.04	8.1 ± 0.96	70.73 ± 6.27	51.89 ± 6.04	3.29 ± 0.70	120.21 ± 10.95
Positive control	9.39 ± 2.40*** (265.37)	2.92 ± 0.89*** (63.45)	37.87 ± 8.46*** (45.13)	29.03 ± 3.48*** (37.41)	1.37 ± 0.35*** (44.76)	64.67 ± 13.39*** (41.85)
Peri-initiation	6.63 ± 2.36* (29.39)	4.41 ± 1.06* (51.03)	50.07 ± 7.53* (32.22)	35.64 ± 4.08** (22.77)	2.00 ± 0.27* (45.99)	86.92 ± 15.57* (34.41)
Postinitiation	5.55 ± 1.69*** (40.89)	4.6 ± 1.02** (57.53)	53.82 ± 10.28** (42.12)	38.91 ± 5.96*** (34.03)	2.16 ± 0.57** (57.66)	93.60 ± 21.02** (44.73)
Peri−post-initiation	4.12 ± 2.04*** (56.12)	5.79 ± 1.31*** (98.29)	61.32 ± 9.48*** (61.92)	42.09 ± 5.04*** (44.99)	2.28 ± 0.57*** (66.42)	107.08 ± 12.95*** (65.58)

Data are presented as mean ± SD. Data in parentheses represent relative percentage change in parameters between normal versus positive control and positive control versus experimental. Treatment schedule of the groups is specified in “Materials and methods” section. Statistical comparison: normal versus positive control and positive control versus experimental
*$p < 0.05$
**$p < 0.01$
***$p < 0.001$
CAT, catalase; GRE, ginseng root extract; GSH, glutathione; LPO, lipid peroxidation; SD, standard deviation; SOD, superoxide dismutase; Vit., vitamin
1) nmol/mg tissue
2) μmol/mg tissue
3) Specific activity in mol/mg protein
4) μmol H_2O_2 consumed/min/mg protein
5) μg/mg tissue
6) mg/g tissue

Table 5
Variations in different biochemical parameters in skin during chemical-induced skin carcinogenesis with or without GRE administration

Treatment group	LPO[1)]	GSH[2)]	SOD[3)]	CAT[4)]	Vit. C[5)]	Total protein[6)]
Normal	2.97 ± 0.80	5.09 ± 1.22	61.07 ± 12.01	43.72 ± 6.29	2.40 ± 0.65	98.48 ± 14.79
Negative control	2.4 ± 0.80	5.57 ± 1.73	62.52 ± 8.68	46.53 ± 5.93	2.85 ± 0.62	103.71 ± 7.19
Positive control	10.5 ± 1.66*** (253.54)	1.45 ± 0.72*** (71.51)	27.6 ± 5.63*** (54.81)	29.03 ± 3.31*** (33.60)	1.18 ± 0.45*** (50.83)	54.87 ± 6.95*** (44.28)
Peri-initiation	8.68 ± 1.14* (17.33)	2.77 ± 0.91** (91.03)	36.54 ± 4.92* (32.39)	35.64 ± 3.38* (22.77)	1.79 ± 0.81* (51.69)	70.16 ± 9.54* (27.87)
Postinitiation	7.73 ± 1.52*** (26.38)	3.31 ± 0.81*** (128.28)	46.71 ± 9.29*** (69.24)	38.91 ± 5.81*** (34.03)	2.18 ± 0.46** (84.75)	72.73 ± 12.77** (32.55)
Peri−post-initiation	5.9 ± 1.37*** (43.81)	4.15 ± 0.70*** (186.21)	56.94 ± 7.64*** (106.30)	42.09 ± 6.73*** (44.98)	2.30 ± 0.51*** (94.92)	107.08 ± 13.58*** (95.15)

Data are presented as mean ± SD. Data in parentheses represent relative percentage change in parameters between normal versus positive control and positive control versus experimental. Treatment schedule of the groups is specified in “Materials and methods” section. Statistical comparison: normal versus positive control and positive control versus experimental
*$p < 0.05$
**$p < 0.01$
***$p < 0.001$
CAT, catalase; GRE, ginseng root extract; GSH, glutathione; LPO, lipid peroxidation; SD, standard deviation; SOD, superoxide dismutase; Vit., vitamin
1) nmol/mg tissue
2) μmol/mg tissue
3) Specific activity in mol/mg protein
4) μmol H_2O_2 consumed/min/mg protein
5) μg/mg tissue
6) mg/g tissue

decrease significantly ($p \leq 0.001$) in the carcinogen-treated control group. The SOD level was significantly increased in the skin and liver, after GRE administration in Group IV ($p < 0.05$; $p < 0.05$), V ($p < 0.001$; $p < 0.01$), and Group VI ($p < 0.001$; $p < 0.001$), respectively. The catalase content was recorded to be significantly ($p < 0.001$) higher in the vehicle-treated control group when compared with the positive control group. Consumption of ginseng extract in experimental Group IV ($p < 0.05$; $p < 0.01$), Group V ($p < 0.001$; $p < 0.001$), and Group VI ($p < 0.001$; $p < 0.001$) restored the catalase activity significantly in both the skin and the liver (Tables 4 and 5).

Further, the ascorbate activity was significantly depleted in the liver ($p < 0.001$) and skin ($p < 0.001$) after the application of a carcinogen and a promoter in Group III, in comparison to the normal group. The oral administration of GRE significantly increased the vitamin C content of the liver and skin in the animals of Group IV ($p < 0.05$; $p < 0.05$), Group V ($p < 0.01$; $p < 0.01$), and Group VI ($p < 0.001$; $p < 0.001$) when compared to Group III. The total protein content was also found to be significantly inhibited in the liver ($p < 0.001$) and skin ($p < 0.001$) of the animals of Group III when compared to normal animals. The GRE intake by oral gavage caused a significant increment in the total protein content in the liver and skin of the mice of Group IV ($p < 0.05$; $p < 0.05$), Group V ($p < 0.001$; $p < 0.01$), and Group VI ($p < 0.001$; $p < 0.001$) in comparison to carcinogen-treated animals (Tables 4 and 5).

3.3. Histopathological study

The animals of the vehicle-treated control group exhibited a uniform arrangement of skin layers, starting from the outer side keratin layer, to the epidermis, dermis, and basal layer (Fig. 2A). This symmetry was found to be disturbed in the carcinogen-treated control animals in the form of hyperkeratosis, thickening of the epidermis called epidermal hyperplasia, erosion of the epidermis, and extension of the epidermis into the dermis (dermal invasion). The sebaceous glands and hair follicles were also severely damaged (Fig. 2B). The tumors of Group III mice exhibited large keratinized

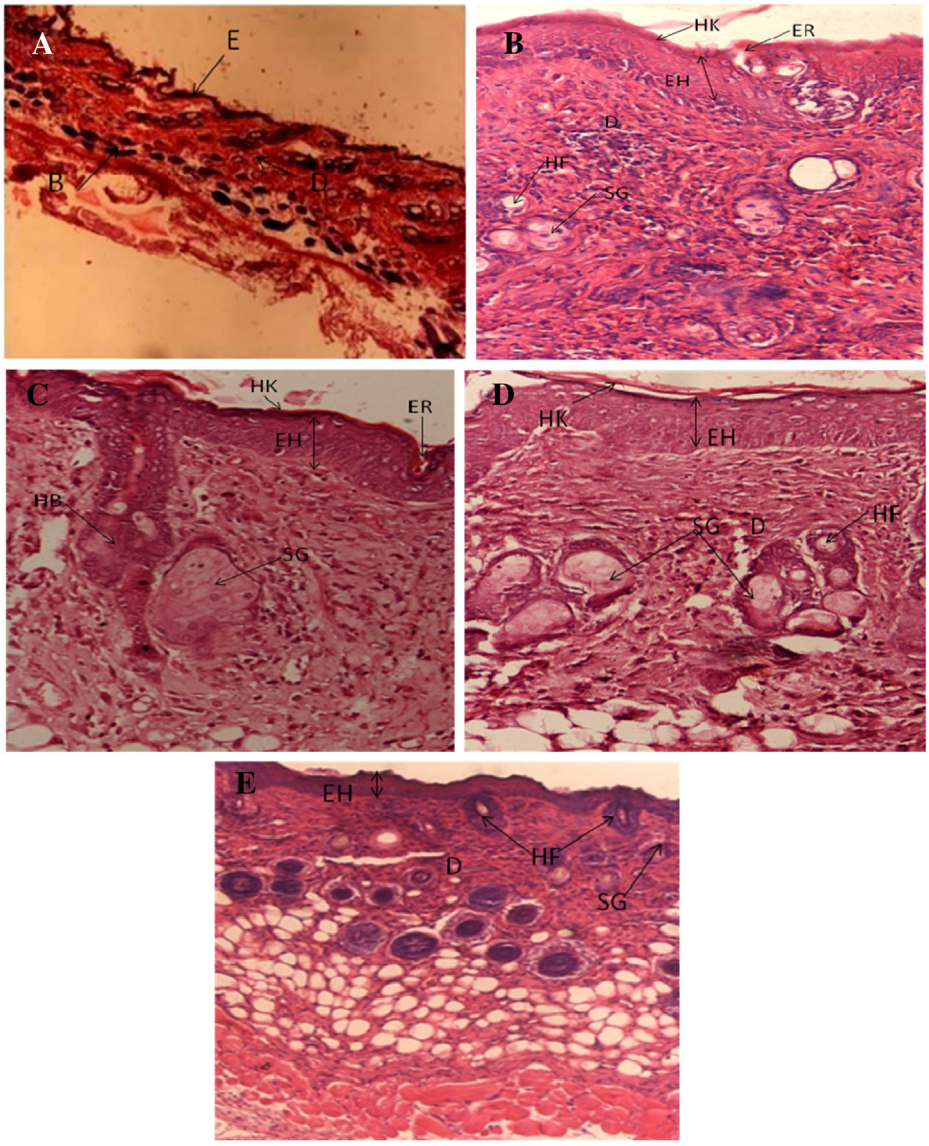

Fig. 2. Photographs (200×) indicating histopathological variations in the skin tissue sections of animals of different groups. (A) Normal group, (B) Group III, (C) Group IV, (D) Group V, and (E) Group VI. D, dermis, EH, epidermal hyperplasia; ER, erosion; HF, hair follicle; HK, hyperkeratosis; SG, sebaceous gland.

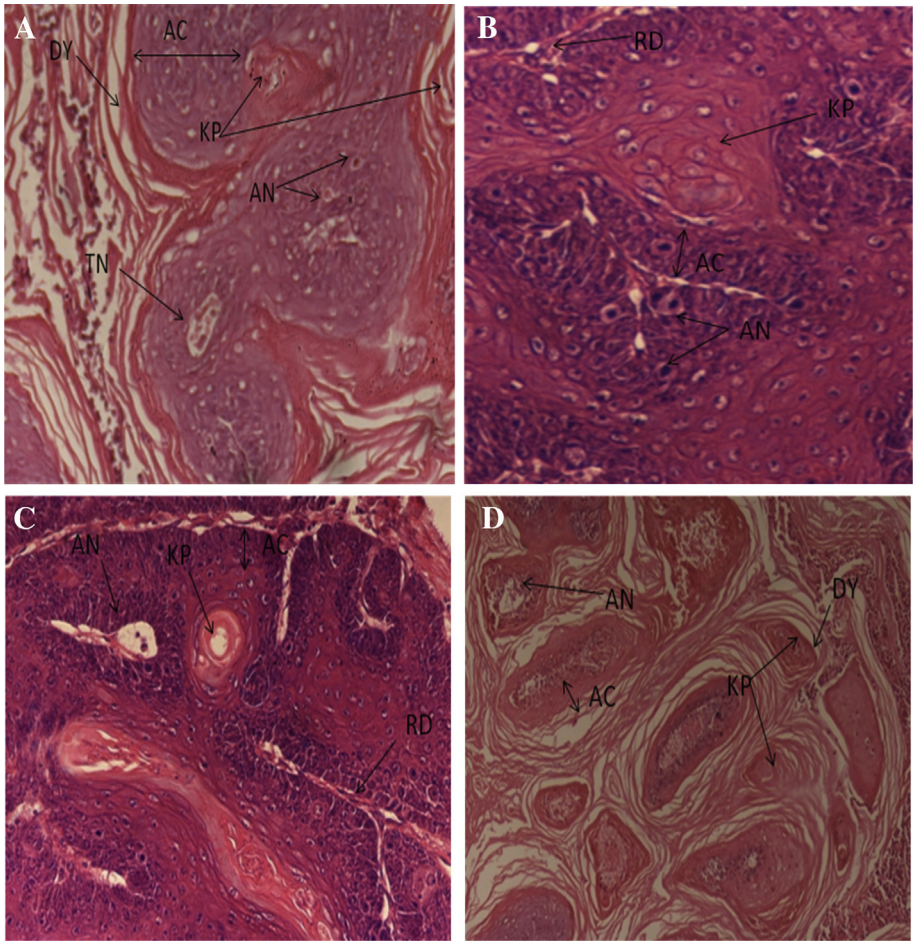

Fig. 3. Photographs (200×) indicating the histological sections of tumors in animals of different groups. (A) Group III, (B) Group IV, (C) Group V, and (D) Group VI. AC, acanthosis; AN, atypical nuclei; DY, dyskeratosis; KP, keratinocyte pearl; RD, reduced stroma; TN, tumor nest.

pearls, acanthosis, reduced stroma, and high infiltration of lymphocytes (Fig. 3A).

After the administration of GRE in Groups IV–VI, the severity of damage to the skin was less. The skin showed reduced hyperkeratosis and epidermal hyperplasia in peri-initiation and post-initiation groups, which were further decreased in Group VI animals (Fig. 2C–E).

The number and size of keratinized pearls, acanthosis, and tumor nests were also noted to be reduced after the GRE treatment in different groups (Fig. 3B–D). Peri- and postinitiation treatment groups (treated with ginseng extract) exhibited a minor disturbance in the skin architecture and small-sized keratinized pearls (Fig. 2E, 3D).

4. Discussion

The continuous rise in cancer incidence has become a challenge for clinicians and scientists. Existing techniques such as chemotherapy and radiotherapy are generally used for cancer treatment, but they are also associated with various limitations and side effects. Therefore, the search for new preventive therapies to reduce the global cancer burden is gaining momentum. Natural dietary supplements have been evidenced to possess many chemopreventive properties due to the presence of antioxidative and anti-inflammatory substances [21].

Roots of *P. ginseng* have bioactive compounds, including a series of triterpenoids and saponins, with steroidal structures similar to those of ginsenosides (Rg3, Rb1, Rc, Re, and Rg1) [22]. Scientific evidence suggests that the presence of Rg3 is associated with the anticarcinogenic potential of GRE [23]. The expression of ornithine decarboxylase, a biochemical marker for tumor promotion, and cyclooxygenase-2, a crucial factor for generating an inflammatory effect, were significantly reduced by Rg3 alone [24,25].

The mouse chemical-induced multistage skin carcinogenesis model is a particularly useful model to examine the genetic and biochemical changes. In the present experiment, the topical application of DMBA was used to initiate carcinogenesis because skin absorption was reported to be the fastest route of entry for these polycyclic aminohydrocarbons. The metabolic activation of DMBA takes place in the liver by Phase-I detoxification enzyme cytochrome P450, which converts it into 3, 4-diol-1, 2-epoxide that covalently binds to DNA and form DNA adducts, ultimately leading to mutation. Croton oil contains 12-O-tetradecanoylphorbol-13-acetate, which is used for skin tumor promotion by the production of reactive oxygen species and hydroperoxides in keratinocytes [26].

In the current study, the animals treated with DMBA/croton oil alone showed 100% tumor incidence, and high tumor yield, tumor burden, and short average latent period due to their carcinogenic potential in the absence of any treatment. After the administration of GRE, a significant reduction occurred in the cumulative number of tumors, and the average latent period was also prolonged.

The consumption of crude plant extracts also showed a significant improvement in all biochemical parameters by restoring them

to normal levels. Free radicals generated by the carcinogen lead to the deterioration of membranes and proteins by the LPO reaction. Various aldehydes, e.g., acrolein, malondialdehyde, and 4-hydroxy-2-nonenal, are formed as secondary metabolites during the peroxidation reaction [27,28]. In the present study, the level of increased malondialdehyde in Group III was associated with the adverse effect of carcinogen, which was found to be reduced during the GRE treatment. Administration of ginseng extract at the peri–post-initiation stage was found to be most effective in reducing malondialdehyde formation during carcinogenesis.

The carcinogen-treated control group was deprived of antioxidants such as GSH, SOD, and catalase because these are consumed during the oxidative stress, but the GRE administration, in the present experiment, normalized the antioxidant content of the cells. Reduced GSH, a tripeptide of amino acids L-glutamate, L-cysteine, and L-glycine, is the most abundant cytosolic thiol and the principal antioxidant used to scavenge free radicals, as well as an essential cofactor to GSH peroxidases and GSH S-transferases for detoxifying nonradical oxidants [29]. Depletion in the GSH levels was reduced in Groups IV–VI after GRE treatment. When ginseng was given throughout the experimental period, it showed best results in comparison to the peri-and postinitiation groups.

SOD is a metalloprotein and chain-breaking antioxidant that converts superoxide radicals into hydrogen peroxide, precluding the generation of reactive oxygen species cascade, including hydrogen peroxide (H_2O_2), hypochlorite (OCl^-), peroxynitrate (ONO_2^-), and hydroxyl radical (HO^-). Further, catalase breaks down H_2O_2 into water and oxygen [30,31]. The augmentation in the SOD and catalase level during the current study demonstrates the antioxidative potential of GRE. The additional dose of antioxidant in the form of GRE helped to restore the levels in the present experiment. Treatment prior to the DMBA application might prevent cancer, but it was more effective when continued till the end of the experiment. Similarly, vitamin C also decimates free radicals in a carcinogenic condition; its level was reduced in Group III, but after the treatment with GRE, it meliorated [32]. The best results were obtained in Group VI due to a continuous supply of GRE as an antioxidant to suppress the adverse effect of carcinogenesis. The outcomes were less effective in Group IV and Group V.

Severe destruction of the skin histology was observed in Group III after the carcinogen treatment alone, and the same results were also noted in previous studies [33,34]. Epidermal hyperplasia and dermal invasion due to hyperproliferation were signs of Squamous Cell Carcinoma (SCC) development. The formation of horny pearls, tumor nests, and acanthosis represented the carcinogenic effect of DMBA/croton oil, which was found to be decreased after the administration of ginseng extract at different stages.

It has been evident that the acidic polysaccharides of *P. ginseng* have a radioprotective effect on bone marrow cells *in vitro* [35]. GRE was also reported to inhibit chromosomal aberrations, intestinal damage, hematological alterations, and depletion of germ cells in the testes during radiation-induced injuries *in vivo* [36–39]. It has been manifested that ginsenosides are the most effective constituents of ginseng for the treatment and prevention of cancer. These ginsenosides are triterpenoid glycosides; a total of 34 kinds of ginsenosides have been isolated from white, red, and fresh ginseng till now. Besides, nonsaponin constituents, e.g., polyacetylenes, sesquiterpenes, alkaloids, aminoglycosides, oligopeptides, etc. [40], of ginseng roots are also attracting attention due to their antioxidative, anticancer, antidiabetic, immunomodulatory, and anticomplementary properties [41]. Types of ginsenosides vary among different parts of a plant, so every part has important medicinal values.

Anticancer properties of ginseng were also previously evaluated for chemical-induced carcinogenesis models, such as benzo[a]pyrene-induced lung adenoma in mice, diethylnitrosamine-induced liver cancer in rats, and azoxymethane-induced colorectal cancer in Sprague–Dawley rats [42–44].

The results obtained from the present study suggest that GRE has the potential to reduce oxidative stress and tumorigenesis, by restoring the antioxidative enzymes, in mammals.

Conflicts of interest

The authors declare that they have no competing interests.

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