RESEARCH COMMUNICATION

Anti-tumour, Anti-mutagenic and Chemomodulatory Potential of *Chlorophytum borivilianum*

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Abstract

In the present investigation anti-tumour, anti-mutagenic and chemomodulatory potential of *Chlorophytum borivilianum* were evaluated. *Chlorophytum borivilianum* root extract had no toxic effect up to a dose of 800 mg/kg body weight/day. Significant increase ($p<0.05$ to $p<0.001$) in the activity of reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) and a significant decrease in the hepatic MDA level were observed at 100, 400, and 800 mg/kg body weight of *Chlorophytum borivilianum* root extract when compared with the control value. Skin papillomagenesis studies demonstrated a significant ($p<0.001$) decrease in cumulative numbers of papilloma, tumour incidence, tumour burden, tumour size and tumour weight and significant ($p<0.01$) increase in average latent period when the animals received *Chlorophytum borivilianum* root extract at a dose level of 800 mg/kg body weight/day orally in double distilled water at pre, peri and post initiation stages of carcinogenesis. A significant reduction in the frequency of chromosomal aberration and micronuclei was observed in the treated animals as compared to carcinogen controls. The present investigation suggests that *Chlorophytum borivilianum* has anti-tumour, anti-mutagenic and chemomodulatory effects.

Keywords: Anti-tumour - anti-mutagenic - chemoprevention - DMBA - croton oil - chlorophytum borivilianum

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Introduction

Chemical carcinogens are basically toxic substances, which are organ specific, target epithelial cells and cause genetic damage (genotoxic).Today, several industrial chemicals have been identified as risk factor for cancer e.g., arsenic, nickel, chromium, vinyl chloride, benzene, napthelamine and polycyclic aromatic hydrocarbons, etc. The role of life style factors such as tobacco and alcohol, in the development of cancer is evident from numerous epidemiological and experimental studies (Weinstein, 1991). Naturally occurring or synthetic chemopreventive agents are also known as anticarcinogens. Plants and plant-products have been in use by humans throughout the world for centuries as traditional cure and as homeopathic remedies for various diseases. In recent years, nutrients as well as non-nutrient phytochemicals are being extensively explored for their potential preventive effects against cancer (Hong and Sporn, 1997; Wattenberg, 1997; Dragsted, 1998; Kelloff et al., 1999).

Chlorophytum borivilianum Linn commonly known as safedmusli belongs to family Liliaceae and found in north eastern India. It is a small perennial herb which is considered to be valuable nervine and general tonic for strength and vigour. The fried root powder is chewed to promote healing of ulcers of the mouth and throat. In Ayurveda the root is used for treating sprue, piles, blood disorder, and as an aphrodisiac and rejuvenator (Nadkarni, 1954; CSIR, 1948-1992; Sahoo and Mudgal, 1995; Yoganarasimhan, 1996). It contains cytotoxic steroidal glycoside saponinchloromaloside-A and spirostanolpentaglycosides embracing beta-D-apiofuranose which are responsible chemicals for anticancer property (Mimaki et al, 1996; Qiu et al. 2000).

In the present study an attempt has been made to evaluate anti-tumour, anti-mutagenic and chemomodulatory potential of *Chlorophytum borivilianum*.

Materials and Methods

Animals

Random bred male Swiss albino mice (7- 8 weeks old) weighing 24 ± 2 gm were used for the experiments. These animals were housed in polypropylene cages in the animal house at temperatures of 24 ± 3°C and feed with standard mice feed (from Hindustan Lever Ltd., India) and tap water *ad libitum*.

Chemicals

7, 12 - dimethylbenz (a) anthracene (DMBA), croton oil, reduced glutathione (GSH), 5,5- dithio-bis-2-nitrobenzoic acid (DTNB), thiobarbituric acid (TBA),

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colchicine and fetal calf serum (FCS) were obtained from Sigma Chemicals Co. (St. Louis, MO. USA). The other chemicals were obtained from local firms and were of the AR grade. DMBA was dissolved in acetone at a concentration of 100 g/50 μl and croton oil was diluted in acetone to give a 1% dilution.

Preparation of Chlorophytum borivilianum extract

Plant material (*Chlorophytum borivilianum* Linn.) was collected locally, identified and the specimen was placed at Herbarium, Department of Botany, University of Rajasthan, Jaipur. The voucher number is RUBL - 19902. Mature roots were washed, air dried in shade, powdered and extracted, with double distilled water. The extract thus obtained was vacuum evaporated to make it in powder form. This extract was redissolved in double distilled water just before oral administration.

Experimental design

Animals were divided into following four groups of 10 animals each to evaluate maximum tolerance dose and chemomodulatory effect of *Chlorophytum borivilianum* root extract.

Group I (n=10) : Animals were fed a normal diet and sham - treated with 100 μl distilled water by oral gavage daily, for 7 days; this group of animals served as control.

Group II a (n = 10): Animals were fed a normal diet and treated with 100 μl of root extract of *Chlorophytum borivilianum* 100 mg/kg body weight per animal per day by oral gavage, for 7 days.

Group II b (n = 10): Animals were fed a normal diet and treated with 100 μl of root extract of *Chlorophytum borivilianum* 400 mg/kg body weight per animal per day by oral gavage, for 7 days.

Group II c (n = 10): Animals were fed a normal diet and treated with 100 μl of root extract of *Chlorophytum borivilianum* 800 mg/kg body weight per animal per day by oral gavage, for 7 days.

Drug Tolerance Study (DTS)

All these animals were observed regularly till 30 days for any sign of morbidity, mortality and behavioural toxicity. A maximum tolerance dose of *Chlorophytum borivilianum* root extracts was determined accordingly.

Biochemical Study

Activity of hepatic reduced glutathione (GSH), catalase (CAT) superoxide dismutase (SOD) and MDA (lipid peroxidation) level were estimated in liver of animals of all four groups.

Lipid peroxidation

Lipid peroxidation in the liver was estimated spectrophotometrically by thiobarbituric acid reactive substances (TBARS) method, as described by Ohkhawa et al.(1979) and is expressed in terms of malondialdehyde (MDA) formed per mg tissue. In brief, 0.8 ml of homogenate was mixed with 0.2 ml of 8.1% Sodium dodecyl sulphate (SDS) to which 1.5 ml of 20% acetic acid was added. Then 1.5 ml of 0.6% TBA was added and placed in a water bath for 1 hr at 80°C, cooled in ice and mixed with 5 ml mixture of n-butanol and pyridine (15:1) centrifuged at room temperature for 10 min at 3,000 rpm. The absorbance of the clear supernatant was measured against blank of distilled water at 532 nm.

Reduced glutathione

Reduced glutathione was estimated as total nonprotein sulphydryl group by the method as described by Moron et al. (1979). Homogenates were immediately precipitated with 0.1 ml of 25% trichloroacetic acid and the precipitate was removed after centrifugation. Free SH groups were assayed in a total 3 ml volume by adding 2 ml of 0.6 mM DTNB prepared in 0.2 M Sodium phosphate buffer (pH8.0), to 0.1 ml of the supernatant and absorbance was read at 412 nm using a UV-VIS Systronics spectrophotometer. GSH was used as a standard to calculate n mole of - SH content / gm tissue.

Superoxide Dismutase (SOD) Assay

The activity of superoxide dismutase (SOD) in liver homogenate was determined by the method of Marklund and Marklund (1974). Animals were killed by cervical dislocation and the entire liver was then perfused immediately with 0.9% NaCl (cold saline).Homogenate (10% w/v) was prepared in 5 ml of double distilled water. Now1.5 ml of 100 mM Tris – HCl buffer, 0.5 ml of 6 mM EDTA, 5 µl tissue homogenate and 1 ml of 0.6 mM pyrogallol solution were added in a cuvette. The rate of auto-oxidation of pyrogallol was taken from the increase in absorbance at 420 nm in a spectrophotometer every minute up to 4 min.

Catalase (CAT) Assay

The activity of catalase (CAT) in liver homogenate was determined according to the method described by Aebi (1984). Animals were killed by cervical dislocation and the entire liver was then perfused immediately with 0.9% NaCl (cold saline).Homogenate (10% w/v) was prepared in 5 ml of double distilled water. The 2 ml of phosphate buffer, 5 μL of erythrocyte lysate/tissue homogenate and 1 ml of 30 mM H_2O_2 were also added in cuvette. Decrease in absorbance every 10 sec interval for 30 sec at 240 nm in a UV spectrophotometer were recorded.

Anti-tumour and anti-mutagenic Study
Induction of tumour

The dorsal skin of all the animals was shaven 3 days before the commencement of the experiment and only those animals in the resting phase of the hair cycle were chosen for the study. For induction of tumours a two stage protocol consisting of initiation with a single topical application of a carcinogen (7, 12 - dimethylbenz (a) anthracene (DMBA) followed by a promoter (croton oil) three times in a week were employed (Berenblum, 1941; Prashar et al., 1994; Qiblawi and Kumar 1999; Panwar et al., 2005).Animals were divided into two groups to investigate anti-tumour and anti-mutagenic potential of *Chlorophytum borivilianum* root extract.

Control Group: A group of 10 animals was treated with DMBA (100 μg/50μl acetone per animal) on day 0 and two weeks later, 0.1 ml croton oil (1% in 100 μl acetone)

was applied topically on the shaven area. This treatment was continued three times in a week till the termination of the experiment.

Treatment Group: A group of 10 animals was administered root extract of *Chlorophytum borivilianum* dissolved in double distilled water (DDW) (800 mg/kg body weight) from 7 days before the application of DMBA (100µg/50µl acetone per animal) and throughout the experimental period. Croton oil (three times in a week) was applied two weeks later till the end of the experiment.

During the 16 weeks of experiments, mice were observed weekly and weighed. The mice were carefully examined once a week for the presence of skin papilloma and the number of papillomas on each affected mice were recorded. Papillomas were defined as a lesion with a diameter greater than 1 mm that persisted for at least two consecutive observations. These animals were also used for anti-mutagenic study by chromosomal aberration analysis and micronuclei assay.

Tumour Study

Cumulative number of papillomas, tumour incidence, tumour burden, weight of tumour and size of tumour were calculated after termination of the experiment. Average latent period was calculated as time lag between the application of the promoting agent and the appearance of tumours in 50% animals (Prashar et al., 1994).

Anti mutagenic study

Cytogenetic damage in the bone marrow cells were studied by chromosomal aberrations and micronuclei induction.

Chromosomal aberration analysis:- Animals were administered (i.p.) with 0.25% colchicine. The animals were sacrificed after two and the bone marrow cells from femur were flushed with 0.56% KCl. Suspension was centrifuged and the pellet was kept in acetic acid-methanol (1:3) for 1 hr and centrifuged. This cycle was repeated thrice. The final cell suspension was dropped on pre-chilled slides, flame dried and stained with 4% Giemsa (Savage, 1975; Bender et al., 1988).

Micronuclei assay

The femurs of mice were dissected out and the bone marrow was flushed out, vortexed and centrifuged. The pellet was resuspended in a few drops of fetal calf serum. Smear was made on a pre-cleaned, dry slides, air dried and fixed in absolute methanol. The slides were stained with May-Grunwald's stain. The micronuclei per 1000 cells were calculated (Schmid, 1975).

Statistical Analysis

Statistical significance of differences between the groups was determined by Student's t-test.

Results

Drug Tolerance Study

No toxic effects were observed in terms of sickness, mortality, morbidity and behaviour in animals treated with different doses (100, 400 and 800 mg/kg body weight/day) of *Chlorophytum borivilianum* root extract (orally) for 7 consecutive days. It suggests that extracts of *Chlorophytum borivilianum* root can be tolerated by mice up to 800 mg/kg body weight/day.

Biochemical study (Figures 1, 2, 3, and 4)

Animals of group II(a) showed non-significant increase in the LPO level (3.3181±0.0492 n mole/mg protein $p<0.50$), non-significant decrease in GSH content (0.0706±0.0033 μ mole/gm tissue $p<0.05$) and significant increase in SOD (2.3414±0.0264 μ mole/mg protein $p<0.005$) and CAT (76.4636±1.1138 μ mole H_2O_2 consumed/min/mg protein $p<0.001$) compared to

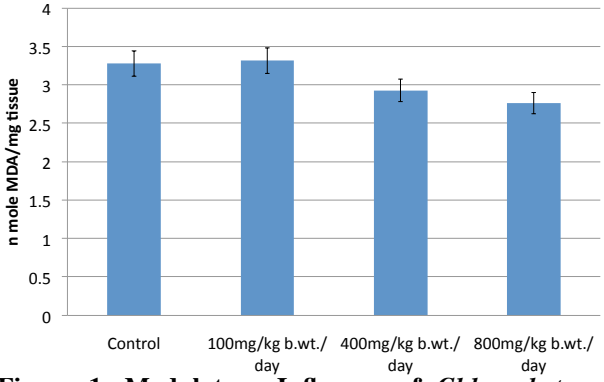

Figure 1. Modulatory Influence of *Chlorophytum Borivilianum* Root Extract at Different Doses on Mice Hepatic Lipid Peroxidation (LPO) Levels

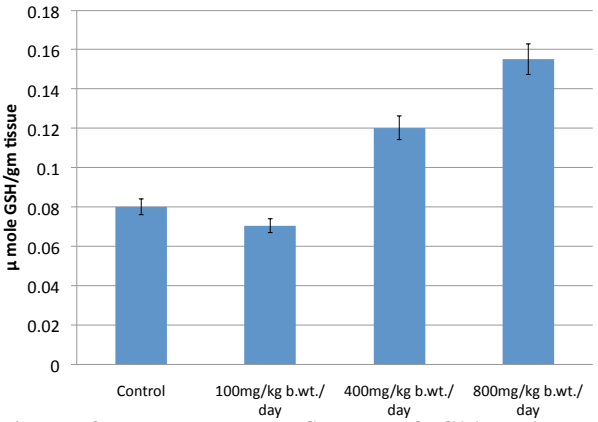

Figure 2. Modulatory Influence of *Chlorophytum Borivilianum* Root Extract at Different Doses on Mice Hepatic Reduced Glutathione (GSH) Contents

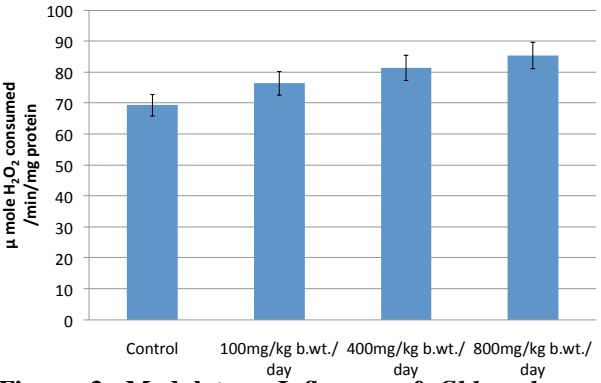

Figure 3. Modulatory Influence of *Chlorophytum Borivilianum* Root Extract at Different Doses on Mice Hepatic Catalase (CAT) Contents

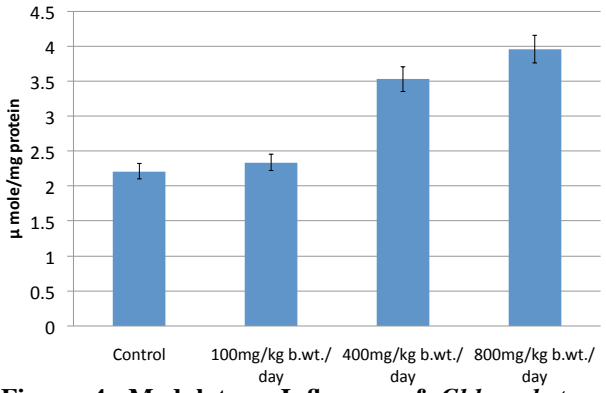

Figure 4. Modulatory Influence of *Chlorophytum Borivilianum* Root Extract at Different Doses on Mice Hepatic Superoxide Dismutase (SOD) Contents

control group.

Animals with dose level of 400 mg/kg body weight/ day (group IIb) showed highly significant decrease in LPO level (2.9318±0.0371 n mole/mg protein p<0.001) and highly significant increase was noticed in GSH content (0.1204±0.0028 μ mole/gm tissue p<0.001), SOD (3.5313±0.0298 μ mole/mg protein p<0.001) and CAT (81.4697±1.0516 μ mole H_2O_2 consumed/min/mg protein p<0.001) with respect to control.

Similarly, animals of group II(c) showed highly significant decrease in LPO level (2.7651±0.0575 n mole/mg protein p<0.001) and highly significant increase was noticed in GSH content (0.1553±0.0074 μmole/ gm tissue p<0.001), SOD (3.9610±0.0254 μmole/mg protein p<0.001) and CAT (85.4517±0.8874 μmole H_2O_2 consumed/min/mg protein p<0.001) as compared to control.

Therefore *Chlorophytum borivilianum* 800 mg/kg body weight per animal per day was found most significant chemomodulatory effect so this dose level was selected for anti-tumour study.

There was highly significant decrease in LPO level of treatment group (2.7651±0.0575) with compare to control (3.2803±0.0434) n mole/mg protein (p<0.001); highly significant increase was noticed in GSH content 0.1553±0.0074 μ mole/gm tissue (p<0.001) where as in control group it was 0.0802±0.0038 μ mole/gm tissue. SOD in treatment group (3.9610±0.0254 μ mole/mg protein) was found to be significantly higher compare to control (2.2150±0.0258μ mole/mg protein) (p<0.001). Similarly CAT was also found higher in treatment Group (85.4517±0.8874 μ mole H_2O_2 consumed/min/mg protein) (p<0.001) as compared to control (69.3684±1.1600 μ mole H_2O_2 consumed/min/mg protein).

Anti-Tumour study (Figures 5, 6, 7, 8, 9, and 10)

Oral administration of *Chlorophytum borivilianum* root extract at pre, peri and post initiation stages of papillomagenesis showed a significant reduction in the tumour incidence i.e. 56.7±3.3 percent (p<0.001) as compared with the control group i.e. 100±0.0 percent. The cumulative number of papillomas i.e. 22±1.15 (p<0.001) during the observation period were significantly less than the control group (43±0.94). The papillomas were subdivided into different categories according to their

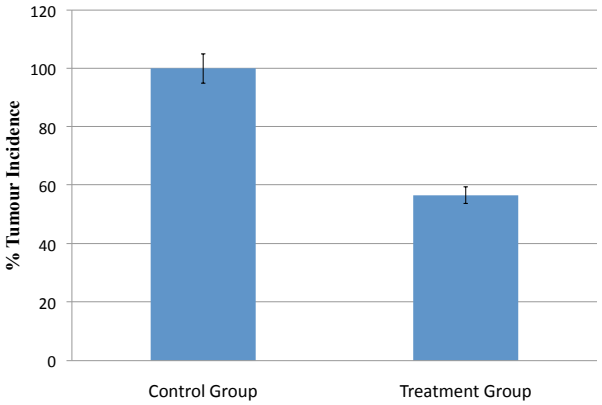

Figure 5. Effect of *Chlorophytum Borivilianum* Root Extract on Tumour Incidence in Treatment Group Compared to Control Group

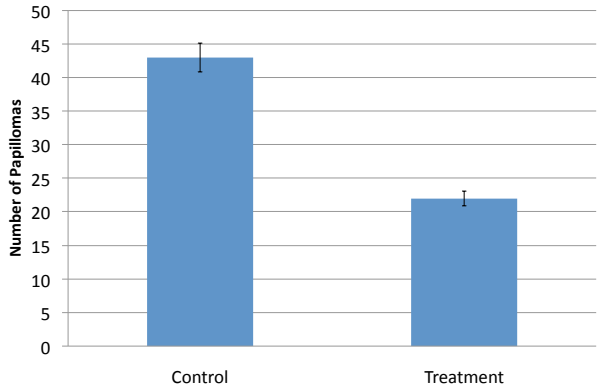

Figure 6. Effect of *Chlorophytum Borivilianum* Root Extract on Cumulative Number of Papillomas in Treatment Group Compared to Control Group

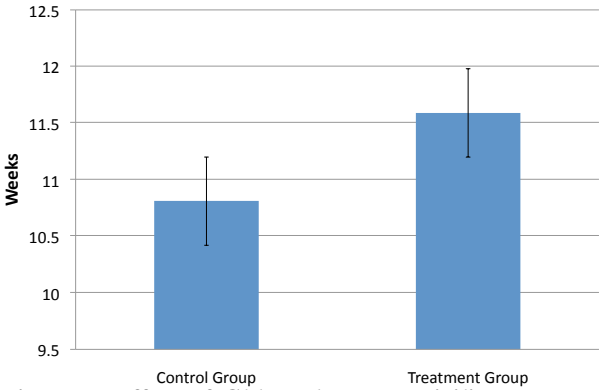

Figure 7. Effect of *Chlorophytum Borivilianum* Root Extract on Average Latent Period in Treatment Group Compared to Control Group

size. The category of <2 mm had 7 (31.8%), 28 (65.1%) papillomas, the 2-6 mm category recorded 13 (59.1%), 11 (25.6%) papillomas and the last category of 6-10 mm developed 2 (9.09%), 4 (9.31%) papillomas in treatment and control group respectively. The average number of tumours per tumour bearing mouse (tumour burden) 3.88±0.11 (p<0.001) was significantly less than the control (4.75±0.10) The average weight of tumour was significantly reduced i.e. 127.33±5.17 mg (p<0.001) in comparison with control group (244.77±7.11 mg). There was less significant increase recorded in the average latent period i.e. 11.59±0.25 (p<0.01) weeks as compared to control group i.e. 10.81±0.10 weeks.

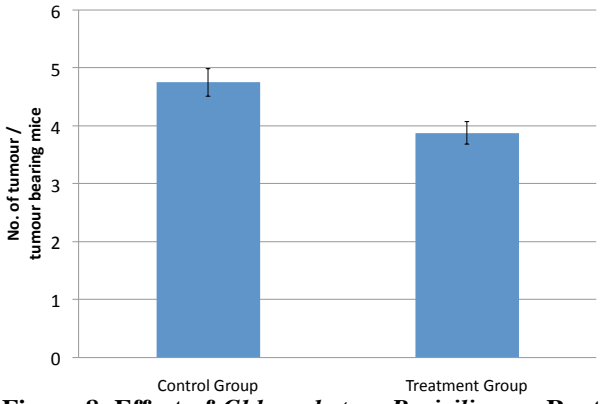

Figure 8. Effect of *Chlorophytum Borivilianum* Root Extract on Tumour Burden in Treatment Group Compared to Control Group

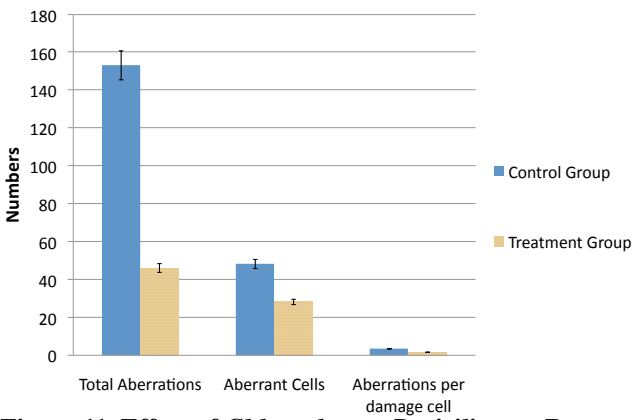

Figure 11. Effect of *Chlorophytum Borivilianum* Root Extract on Frequency of Chromosomal Aberration in Treatment Group Compared to Control Group

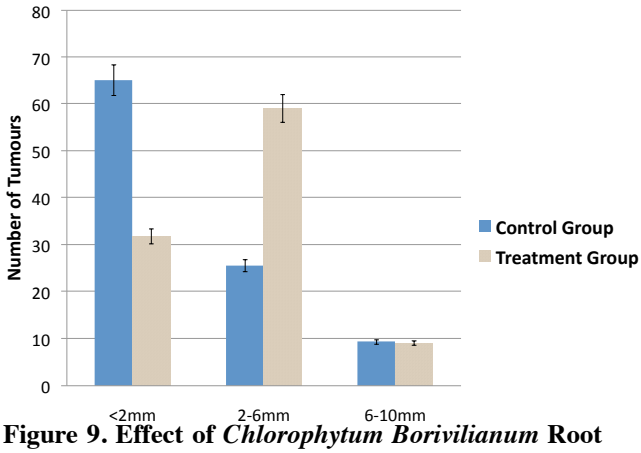

Figure 9. Effect of *Chlorophytum Borivilianum* Root Extract on Size of Tumour in Treatment Group Compared to Control Group

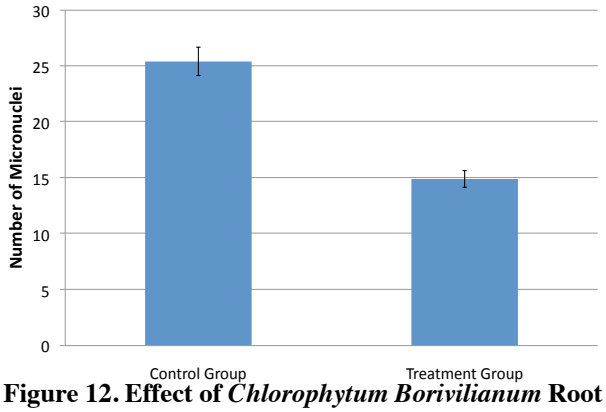

Figure 12. Effect of *Chlorophytum Borivilianum* Root Extract on Frequency of Micronuclei in Treatment Group Compared to Control Group

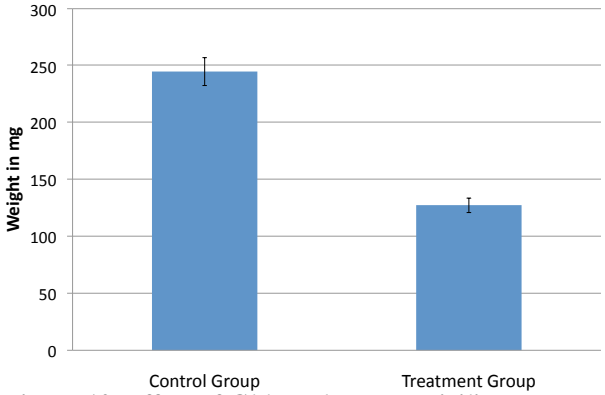

Figure 10. Effect of *Chlorophytum Borivilianum* Root Extract on Average Weight of Tumour in Treatment Group Compared to Control Group

Anti-mutagenic study (Figures 11 and 12)

Animals treated with *Chlorophytum borivilianum* root extract at pre, peri and post initiational stages of papillomagenesis showed significant reduction in the total chromosomal aberrations (46.11±0.57) ($p<0.001$)) in the form of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges, acentric fragments, pulverized cells and polyploids as compared to control (153.14±1.05). Aberrant cells (28.2±0.16) ($p<0.001$) were recorded to be decreased in this group as compared to control group (48.3±0.57). Aberrations/damaged cells (1.63±0.09) ($p<0.005$) were also found to be decreased in this group as compared to control group (3.43±0.20). Animals of this group showed a significant decrease in the number of micronuclei (14.91±0.57) ($p<0.001$) as compared to control (25.43 ± 0.89).

Discussion

Cancer chemoprevention involves the use of either natural or synthetic compounds to delay inhibit or reverse the development of cancer in normal or pre-neoplastic conditions (Pezzuto, 1997). Comprehensive reviews provide strong evidence that high intake of vegetables and fruits are associated with reduced cancer incidence (Steinmetz and Potter, 1991; Block et al., 1992). It is known that balance between Phase I and Phase II enzymes can afford protection against numerous chemical carcinogens, and the induction of antioxidant enzymes facilitates their degradation from the body (Miller, 1978). Regular consumption of fruits and vegetables is associated with reduced risk of cancer (Russo et al., 2005). In our laboratory plant extracts such as Ocimum (Prashar and Kumar, 1995), Spirulina (Mittal et al., 1998), Brassica (Qiblawi and Kumar, 1999), Mentha (Kumar et al., 2005), Ginseng (Panwar et al., 2005a & b), combination of Mentha and Brassica (Sharma and Kumar, 2006), Tribulus (Kumar et al., 2006) and Acacia (Meena et al., 2006) has been proved to be cancer chemopreventive. In the present

investigation it was observed that tumour burden, tumour incidence, cumulative number of papillomas, tumour weight and tumour size has significantly decreased in *Chlorophytum borivilianum* root, extracts treated (at a dose of 800 mg/kg body weight) group as compared to control group (in which only DMBA and croton oil was given, no extract). Average latent period was found to be significantly increased in all treated group as compared to control group.

All these observations are reflection of the chemopreventive activity of aqueous extracts of *Chlorophytum borivilianum* root, and their continuous treatment not only lowers the carcinogenic ability of 7,12-dimethylbenz(a)anthracene but also modulates the effects of the promoter i.e. croton oil.

It is widely believed that inhibition of tumour promotion is a better strategy in cancer chemoprevention than inhibition of tumour initiation because initiation is a short irreversible event whereas promotion is a long cumulative process that is reversible during the initial stage (Agarwal and Mukhtar, 1991; DiGiovanni, 1992). Our results provide additional support for this strategy as well as for the already existing epidemiological and laboratory studies (Perchellet and Perchellet, 1989; Morse and Stoner, 1993; Zheng et al., 1993; Kelloff et al., 1994 & 1996; Brit et al., 1996; Mukhtar and Agarwal, 1996; Boone et al., 1997; Conney et al., 1997; Goodman, 1997; Hong and Sporn, 1997; Lipkin, 1997; Wattenberg, 1997; Arii et al., 1998; Dragsted, 1998). Antioxidant potential of Chlorophytum tuberosum has been investigated for their ability to scavenge 1,1-diphenyl picryl hydrazyl (DPPH), nitric oxide radical along with their capacity to reduce lipid peroxidation in rat liver homogenate, chelation of ferrous ion, radical scavenging potential using chemiluminescence and their total antioxidant capacity (Narasimhan et al., 2006a & b).

The efficacy of *Chlorophytum borivilianum* root in modulating the hyperlipaemic or hypercholesteraemic conditions in male albino rats has been reported. Administration of *C. borivilianum* to hypercholesteraemic rats significantly increased high-density lipoprotein-cholesterol levels and decreased plasma and hepatic lipid profiles. Furthermore, the hypercholesteraemic rats treated with C. borivilianum also exhibited increase in superoxide dismutase and ascorbic acid levels. Administration of *C. borivilianum* root powder also increased the activities of anti-oxidant enzymes and vitamin C levels, which may have enhanced the anti-oxidant capacity of the liver (Visavadiya and Narasimhacharya, 2007). The findings of our studies on the hepatic biotransformation enzymes have demonstrated that prolonged oral treatment to mouse for 7 days with *Chlorophytum borivilianum* root extracts at the dose of 800 mg/kg body weight/day. *C. borivilianum* enhanced the specific level of reduced glutathione, Superoxide dismutase and Catalase and decreased the level of lipid peroxidation after oral administration of *Chlorophytum borivilianum* root for 7 days at the dose level of 800 mg/kg body weight/day.

Evidences have accumulated to suggest that reactive oxygen species (ROS) play an important role in tumour initiation by enhancing or facilitating the metabolic activation and/or initiating effects of carcinogens (Athar, 2002). Because reactive oxygen species have been implicated in premature skin aging, carcinogenesis, DNA damage, activation of signal transduction pathways related to growth differentiation and cell death, it is assumed that antioxidants could act as potential anticarcinogens at multiple stages of skin carcinogenesis (Gupta and Mukhtar, 2002).

The elevated level of GSH protects cellular proteins against oxidation through glutathione redox cycle and also detoxifies reactive oxygen species directly and/or neutralizes reactive intermediate species generated from exposure to xenobiotics including chemical carcinogens (Ketterer, 1998). GSH has been endowed with an important function in maintaining the reduced state of cellular environment, in addition to its conjugating ability owing to nucleophilic center and its involvement in detoxification of xenobiotics that cause toxicity and carcinogenicity. Such a mechanism would decrease the level of reactive electrophiles available to bind DNA, reducing the likelihood of DNA damage and possible induction of carcinogenic process (Seo et al., 2000).

GSH can react chemically with singlet oxygen, superoxide and hydroxyl radicals and therefore function directly as a free radical scavenger. GSH may stabilize membrane structure by removing acyl peroxides formed by lipid peroxidation reactions (Price et al., 1990).

Because of the timing of *C. borivilianum* administration, the modulation of xenobiotic enzyme activity may be more likely to act on reactive species produced during the promotion phase of carcinogenesis rather than metabolites of DMBA itself. Reactive Oxygen Species (ROS) formed during DMBA metabolism or secondarily during tumour formation can diffuse from the site of generation to other targets within the cells or even propagate the injury to other intact cells. DMBA and its metabolites are documented to mediate their mutagenic and carcinogenic effect via ROS generation that acts complementary to the mutation induced by diolepoxides (Rubin, 2001). Thus, the enhanced hepatic LPO in DMBA treated animals may be due to the generation of ROS exacerbated by decreased efficiency of host antioxidant defence mechanisms. The liver, which is rich in GSH, supplies this antioxidant to various extra hepatic tissues via a distinct GSH transport system (Locigno and Castronovo, 2001). GSH maintains the integrity of the liver when the organ is challenged by a wide variety of xenobiotics, ROS and toxic compounds (Lu, 1999). The depletion of GSH resulting from increased utilization to scavenge lipid peroxides may shift the redox status towards oxidative stress. The GSH content in liver tissues observed in present study was significantly elevated suggesting a protective role of *C. borivilianum*.

SOD and CAT play an important role in the detoxification of reactive oxygen species such as O_2^-, OH^-, and H_2O_2, which are involved in genotoxicity, and various stages of chemical carcinogenesis (Lind et al., 1982; Cerutti, 1985; Oberley and Oberley, 1986). Thus, SOD and CAT activities help in maintaining the physiological level of oxygen and hydrogen peroxide by dismutation of oxygen radicals and decomposition of hydrogen peroxide (Gonzales et al., 1984) and hence offer

a protective role against free radicals. Thus, increase in GSH, SOD and CAT activity in conjugation with decrease in LPO caused by menthol may antagonize any cellular injury induced by ROS.

DMBA produced a change in antioxidant enzyme levels in the body. The values of SOD and CAT were significantly higher in the *C. borivilianum* root extracts treated animals. DMBA causes increases in the levels of superoxide radicals and the increased SOD activity seen in the *C. borivilianum* root extracts treated animals would allow the elimination of these DMBA induced superoxide radicals. As a result of dismutation, highly reactive H_2O_2 is formed which was degraded by CAT by converting H_2O_2 to H_2O and O_2.

Treatment with *Chlorophytum borivilianum* root effectively reduced the frequency of DMBA-induced bone marrow micronuclei as well as the extent of hepatic LPO and enhanced the antioxidant status. Elevated levels of GSH protect cellular proteins against oxidation through the glutathione redox cycle and also detoxify ROS directly and/or neutralize reactive intermediate species generated from exposure to xenobiotics, including chemical carcinogens (Ketterer, 1998).

Results of the present study suggest that *Chlorophytum borivilianum* root have modulatory influences on the two-stage skin carcinogenesis and exhibits chemopreventive, antioxidative and antimutagenic activities, which may be due to immunomodulatory and pharmacological properties of these plants.

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