Int J Biol Med Res. 2010; 1(4): 158-164

Contents lists available at BioMedSciDirect Publications

International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com

Original Article

Chemopreventive effect of *Phyllanthus niruri* on DMBA induced skin *papillomagenesis* in swiss albino mice

Priyanka Sharma[a], Jyoti Parmar[a], Preeti Verma[a], Priyanka Sharma[a], P.K.Goyal [a], *

[a], *Professor, Radiation & Cancer Biology Laboratory, Department of Zoology, University of Rajasthan, Jaipur-302 004, India.*

ARTICLE INFO

Keywords:
Carcinogenesis
Catalase
Chemoprevention
Glutathione
Lipidperoxidation
Phyllanthus niruri

ABSTRACT

Phyllanthus niruri is a useful medicinal weed for herbalist and it holds a reputed position in both Ayurvedic and Unani system of medicine. The aim of this work was to evaluate the chemopreventive effect of hydro-alcoholic extract of the whole plant of *Phyllanthus niruri*, in 7-9 weeks old male Swiss albino mice, on two stage process of skin carcinogenesis induced by a single topical application of 7, 12-dimethylbenz (a) anthracene (DMBA) and two weeks later promoted by repeated application of croton oil till the end of experiment (i.e. 16 weeks).The oral administration of *P.niruri* at 1000 mg/kg/b.wt./day during peri and post-initiational phases of papillomagenesis showed significant reduction in tumor incidence, tumor yield, tumor burden and cumulative number of papillomas as compared to carcinogen treated control. The average latent period significantly increased from 7.93 weeks in the control group to 10.52 weeks in the *Phyllanthus niruri* administrated group. Furthermore, a significant increase in reduced glutathione($p<0.001$), catalase ($p<0.001$) and protein ($p<0.001$) level in skin was observed in the P.niruri administered groups as compared to carcinogen treated control, whereas MDA formation in lipid peroxidation was inhibited significantly by *P.niruri* extract ($p<0.001$). The results from the present study suggest the chemopreventive effect of *Phyllanthus niruri* in DMBA induced skin papillomagenesis in Swiss albino mice.

1. Introduction

Cancer is a group of diseases with similar characteristics. Cancer can occur in all living cells in the body and different cancer types have different natural history. Epidemiological studies have shown that 70-90% of all cancers are environmental. Lifestyle related factors are the most important and preventable among the environmental exposures. Non-communicable diseases including cancer are emerging as major public health problems in the world. These diseases are lifestyle related, have a long latent period and needs specialized infrastructure and human resource for treatment. The risk factors of the major non-communicable diseases are tobacco, dietary habits, inadequate physical activity and alcohol consumption. This offers the prospect for integrated primary prevention strategies.

Chemical carcinogenesis in murine and possibly human skin is a multi step process including initiation, promotion and progression [1, 2]. In mouse skin, a single topical application of 7,12-dimethylbenz [a] anthracene (DMBA) effects initiation while promotion is accomplished by repeated topical applications of a promoter such as 12-O-tetradecanoylphorbol-13-acetate (TPA) [3,4]. In contrast to initiation, this is irreversible and possibly unavoidable because of continuous exposure to chemical and physical carcinogenic agents, the process of promotion is reversible [4,5].

The reversibility of tumor promotion therefore provides an opportunity to interrupt or delay the development of altered lesions resulting in tumor formation. One approach to cancer chemoprevention involves the administration of natural and synthetic nutrient or non-nutrient compounds in order to examine their potential role in the prevention of initiational and/or promotional stages of carcinogenesis [6]. A number of these chemopreventive agents are capable of modulating the biotransformation system enzymes, which play an important role in the activation, conjugation and subsequent excretion of xenobiotics including carcinogens [7]. Some epidemiological studies show a protective effect of vegetables and fruits against cancer [8]. An attractive hypothesis is that vegetables and fruits contain compounds that have a protective effect independent of that of known nutrients and micronutrients. This is supported by

* Corresponding Author : Dr. P.K.Goyal,
Professor, Radiation & Cancer Biology Laboratory,
Department of Zoology, University of Rajasthan, Jaipur-302 004, India.
Phone: 09829134133, Fax No. : 0091 141-2656273
E-mail: pkgoyal2002@rediffmail.com; priya.shiv04@gmail.com

in vitro and in vivo studies, which show that naturally occurring plant compounds may inhibit various stages in the cancer process. In this particular context, phytochemicals and natural products containing antioxidants, has been suggested for cancer prevention.

It has now been established that the plants, which naturally synthesize and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, and volatiles oils and contain minerals and vitamins, possess medicinal properties. Among such plants, *Phyllanthus niruri* is a useful medicinal plant belongs to family *Euphorbiaceae* commonly known as bhoomi amalaki, stone breaker and *Chanca piedra.* It is a small, erect, annual herb that grows 50-60 cm in height. It is indigenous to the rainforests of the Amazon and other tropical areas throughout the world, including the Bahamas, southern India and China. It grows and spread freely much like a weed. It is a rich source of phytochemicals, including many which have been found only in the *Phyllanthus genus*. Many of the "active" constituents are attributed to biologically active lignans, glycosides, flavonoids, alkaloids, ellagitannins, and phenylpropanoids found in the leaf, stem, and root of the plant.

Phyllanthus niruri is used for so many purposes in herbal medicine systems; in clinical research over the years, the plant has demonstrated anti-hepatotoxic, [9] antilithic, [10] analgesic, [11]hypotensive,[12] antispasmodic, [13]antiviral, [14]antibacterial, [15] diuretic, [16] anti-mutagenic, [17]hypoglycemic,[18] hepatoprotective and hypocholesterolemic activities. *Phyllanthus niruri* is a perfect example of a highly beneficial medicinal plant which deserves of much more research. Therefore, the present study has been designed to assess the in vivo chemopreventive efficacy of *Phyllanthus niruri* extract during the peri-initiation and/or during the tumor promotion stage and determining the mechanistic role that biotransformation system enzymes may have in modulation of skin papillomagenesis pattern.

2. Materials and Methods

2.1. Animals care & handling

The animal care and handling was approved by our institution and was done according to guidelines set by the World Health Organization, Geneva, Switzerland, and the Indian National Science Academy, New Delhi, India. The study was conducted on random-breed male Swiss albino mice (7-9 weeks old) weighing 24 ± 2 gm. These animals were housed in polypropylene cages in the animal house under controlled conditions of temperature (25°C ± 2°C) and light (14 light: 10 dark). The animals were fed a standard mouse feed procured from Aashirwad Industries, Chandigarh (India), and water ad libitum. Four animals were housed in one polypropylene plastic cage containing saw dust (procured locally) as bedding material. As a precaution against infections, tetracycline hydrochloride water was given to these animals once each fortnight.

2.2.Chemicals

The initiator, 7, 12-dimethylbenz [a] anthracene (DMBA) and the promoter croton oil were procured from Sigma Chemicals Co., St Louis, and 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.

2.3. Preparation of *Phyllanthus niruri* Extract (PNE)

The whole plant P. niruri was collected after proper identification (Voucher No. RUBL 20247) by a competent botanist from the Herbarium, Department of Botany, University of Rajasthan, Jaipur. The whole plant was powdered in a mixture and the hydro-alcoholic extract was prepared by refluxing with the double distilled water (DDW) and alcohol (3:1) in a round bottom flask for 36 hrs at 60ºC. The liquid extract was filtered, cooled and concentrated by evaporating its liquid contents in oven and collected. The powdered extract, termed Phyllanthus niruri extract (PNE), was redissolved in DDW prior for the oral administration in mice. The required dose for treatment was prepared by dissolving the extract in DDW at a dose level of 1000 mg/kg body weight.

2.4. Experimental Design

A total of 40 animals were randomly divided into the following 4 groups to evaluate the anticarcinogenic potential of *P.niruri* extract against DMBA-induced skin papillomagenesis in mice. The dorsal skin of the animals in the back area was shaven 3 days before the commencement of the experiment and only those animals in the resting phase of hair cycle were chosen for the study.

Group I: Vehicle treated control / Normal (n = 10) - Animals of this group received topical application of acetone (100µl/ mouse) on the shaven dorsal skin and double distilled water (equivalent to PNE i.e.100µl / mouse) given by oral gavage for 16 weeks.

Group II: PNE treated control / Drug alone (n = 10) - Animals of this group were put on a normal diet and administered *Phyllanthus niruri* extract at a dose of 1000 mg/kg/b. wt. /day orally once in a day for 16 weeks study period.

Group III: Carcinogen treated Control (n = 10) These animals were treated with a single dose of DMBA (100 µg/100 µl of acetone) over the shaven area of the skin of the mice. Two weeks later, croton oil (1% in 100 µl of acetone) was applied as a promoter 3 times per week until the end of the experiment (i.e., 16 weeks).

Group IV: PNE treated Experimental (n = 10) Animals of this group were administered *P. niruri* extract (1000 mg/kg/b. wt./animal/day) by oral gavage starting from 7 days before of DMBA application and continued until the end of experiment (i.e., 16 weeks).

The following studies were performed.

2.5. Tumor study

During the 16 weeks of experimentation, mice were observed daily and weighed weekly. Papillomas appearing on the shaven area of the skin were examined and recorded at weekly intervals in all the above groups. Only those papillomas which persisted for two weeks or more, with a diameter greater than 2 mm, have been taken into consideration for final evaluation of the data. Skin papillomas, which regressed after one oberservation, were not considered for counting.

The following parameters were taken into consideration:

i. Cumulative number of papillomas: The Cumulative number of papillomas appeared till the termination of the experiment.

i. Tumor incidence: The number of mice carrying at least one tumor, expressed as a percentage incidence.

ii. Tumor yield: The average number of papillomas per mouse.

iii. Tumor burden: The average number of tumors per tumor bearing mouse.

iv. Diameter: The diameter of each tumor was measured.

v. Weight: The weight of the tumors of each animal at the termination of each experiment was measured.

vi. 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 week by the time in weeks after the application of the promoting agent and dividing the sum by total number of tumors.

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

Where F is the number of tumors appearing each week, X is the numbers of weeks, and n is the total number of tumors.

2.6.Biochemical Study

Biochemical alterations were studied in animals of all the groups at the time of the termination of the experiment (i.e., the 16th week). At the end of the 16th week, the animals were killed by cervical dislocation and dorsal skin affected by tumors was quickly excised and washed thoroughly with chilled 0.9% NaCl (pH 7.4). The tissue (skin) was then weighed and blotted dry. A 10% tissue homogenate was prepared from part of the sample in 0.15 M Tris-KCL (pH 7.4), and the homogenate was then centrifuged at 2500 rpm for 10 minutes. The supernatant thus obtained was taken for estimation of reduced glutathione (GSH) and lipid peroxidation (LPO).

2.7.GSH estimation

The level of GSH was estimated as total nonprotein sulphydryl group by the method of Moron et al (1979) [19]. Homogenate were immediately precipitated with 100µL of 25% TCA and the precipitate was removed after centrifugation. Free endogenous-SH was assayed in a total volume of 3 ml by the addition of 2 ml of 0.6 mM 5, $5^{\cdot}$ dithio-bis (2-nitrobenzoic acid) (DTNB) prepared in 0.2 M phosphate buffer (pH 8.0) to 0.1 ml of the supernatant 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 are expressed as µmole GSH/gm tissue.

2.8.LPO estimation

The level of LPO was estimated spectrophotometrically by thiobarbituric acid reactive substances (TBARS) method, as described by Ohkhawa et al (1979) [20]. Briefly, thiobarbituric acid (0.6%), sodium dodecyl sulphate (0.1%), and trichloroacetic acid (20%) were added to 0.2 ml of the tissue homogenate 10% prepared as described above. This mixture was heated for 60 minutes, cooled, and extracted with N-butanol-pyridine (15:1), and centrifuged. The optical density (OD) of LPO was recorded at 532 nm. The content of LPO is expressed as n mole/mg of tissue.

2.9.Catalase Assay

The catalase was assayed by the method of Aebi [21]. The change in absorbance was followed spectrophotometrically at 240 nm after the addition of H_2O_2 (30 mM) to 100 µl of the supernatant (10% of skin homogenate prepared in 50 mM phosphate buffer and centrifuge for 10 min.) in 50 mM phosphate buffer (pH 7).The activity of the enzyme was expressed as U/mg of tissue, where U is µ mole of H_2O_2 reduced/min.

2.10.Protein estimation

Protein estimation was determined by the method of Lowry et al (1951) using bovine serum albumin as a standard [22].

2.11.Statistical Analysis

The results are expressed as the mean ± standard error. The data from biochemical determinations were analyzed using the Student's t- test.

3. Results

The findings of the present study are furnished in Tables 1 and figure 1-8.The gain in the body weight in mice was not affected either by the carcinogen or by phyllanthus extract (PNE) administration. A gradual increase in body weight was noted in all animal groups that were near to normal values of the vehicle treated control animals. The vehicle treated control animals (Group I) as well as the PNE alone administered (Group II) did not show any tumor incidences.

In the PNE treated experimental group IV, in which *P.niruri* extract was given by oral gavage at a dose of 1000 mg/kg. body wt./animal, mice showed a significant decrease in tumor number, diameter and weight as compared with that of the carcinogen control group. In the carcinogen treated control (group III), in which a single topical application of DMBA was followed 2 weeks later by repeated application of croton oil, skin papillomas appeared in all the animals (100 % tumor incidence) from week 7 onwards. The cumulative number of papillomas as induced during the observation period of 16 weeks was 62. The tumor yield as well as the tumor burden was found to be 6.2.

Mice of group IV, given a continuous treatment of *P.niruri* orally at peri- and post initiational phases, showed a significant reduction in the incidence of tumors (40 %) as well as the cumulative number of papillomas (19), tumor yield (1.9) and tumor burden (4.7) (Fig. 1-4) as compared to carcinogen treated control group. The results also indicate that *P.niruri* could prolong the average latency period of tumor occurrence. The latency period was found to be 7.93 week in the carcinogen treated control group, whereas it was significantly higher i.e. 10.52 weeks in PNE experimental group.

A significant increase in glutathione ($P < 0.001$), catalase ($P < 0.001$) and protein ($P < 0.001$) level was noted in the skin of PNE administered animals (Group IV) than the carcinogen treated control animals (Group III) (Fig. 6-8). On contrary, the formation of malondialdehyde, measured as index of lipid peroxidation level, revealed a significant ($P < 0.001$) decrease in *P.niruri* administered animals (Group IV) as compared to carcinogen control (Group III) (Fig. 5).

Table 1. Chemopreventive effect of P. niruri extract (PNE) against DMBA-induced skin tumorigenesis in mice*

Treatment Group	Body Weight (g)		Tumor Size (mm)		Tumor weight (gm)	Average latent period (Weeks)
	Initial	Final	2-5 mm	6-9 mm		
Group I Normal	25.52±1.72	33.20±1.24	-	-	-	-
Group II PNE alone	25.96±1.73	33.17±1.03	-	-	-	-
Group III Carcinogen control	25.60±1.64	30.81±2.29	44	18	1.672	7.93
Group IV PNE experimental	24.63±1.03	32.19±1.25	16	3	0.528	10.52

*Treatment schedule of the groups is specified in materials and methods.

Figure -1. Variation in the tumor incidence during DMBA-induced skin tumorigenesis with or without P.niruri extract administration.

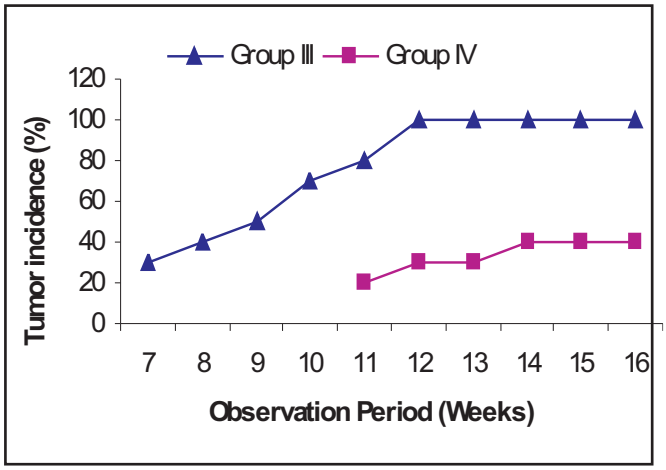


Figure -2. Variation in the cumulative frequency during DMBA-induced skin tumorigenesis with or without P.niruri extract administration.

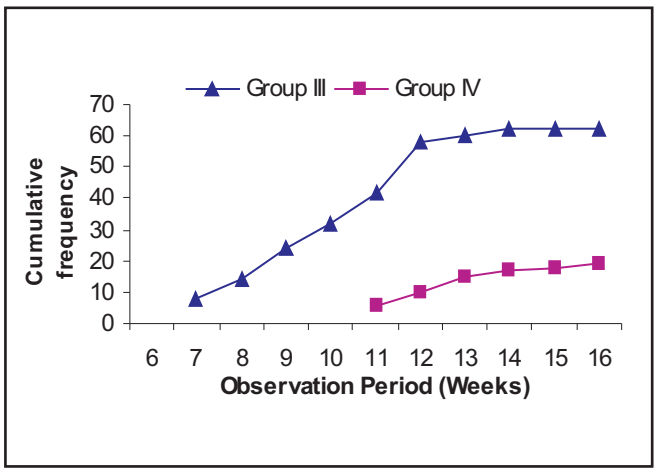


Figure -3. Variations in the tumor yield during DMBA-induced skin tumorigenesis with or without P.niruri extract

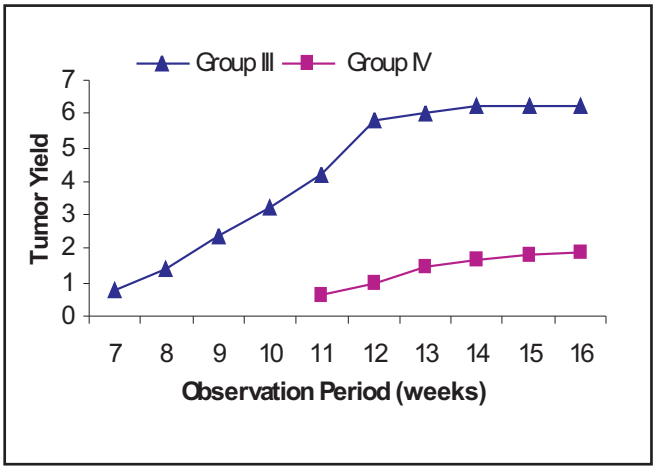


Figure- 4. Variations in the tumor burden during DMBA-induced skin tumorigenesis with or without P.niruri extract administration.

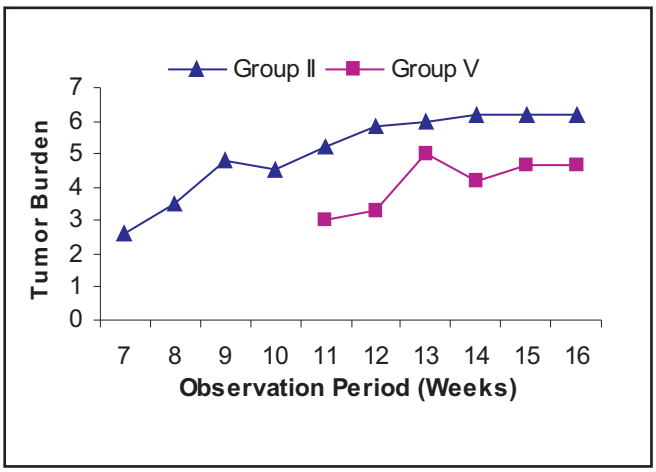


Figure- 5. Variations in the lipid peroxidation (LPO) level in skin during DMBA-induced skin tumorigenesis with or P.niruri extract administration *p≤0.001

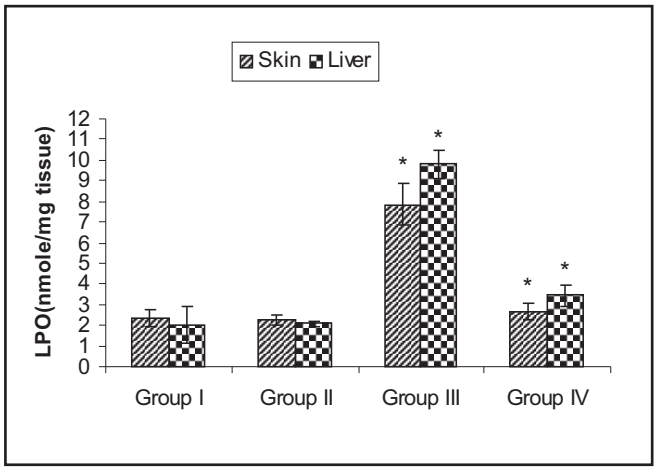


Figure-6. Variation in the glutathione (GSH) level in skin during DMBA-induced skin tumorigenesis with or without P.niruri extract administration *p≤0.001

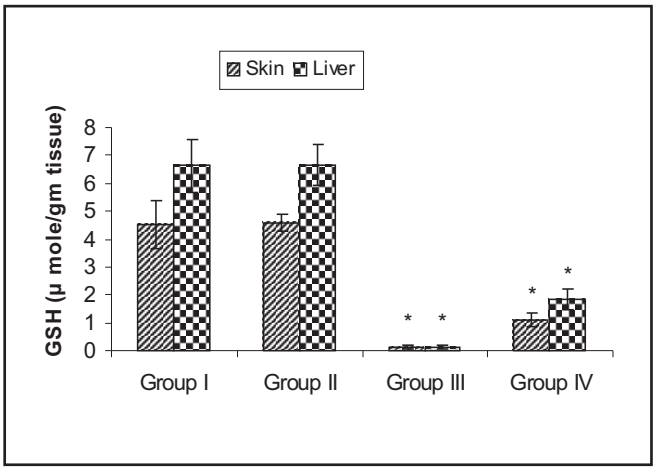


Figure -7. Variations in the catalase (CAT) level in skin during DMBA-induced skin tumorigenesis with or Without P.niruri extract administration *p≤0.001

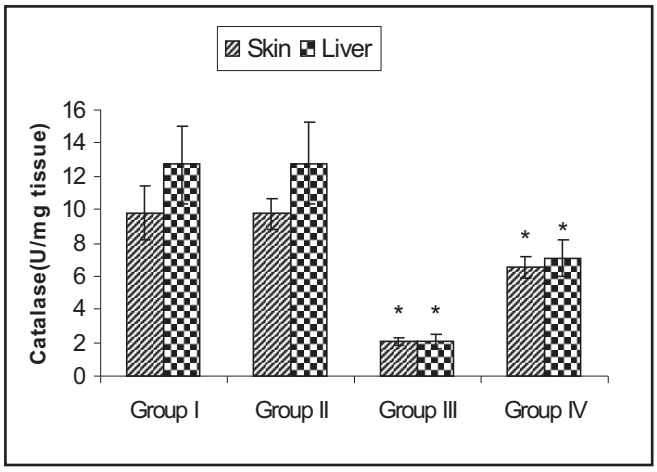
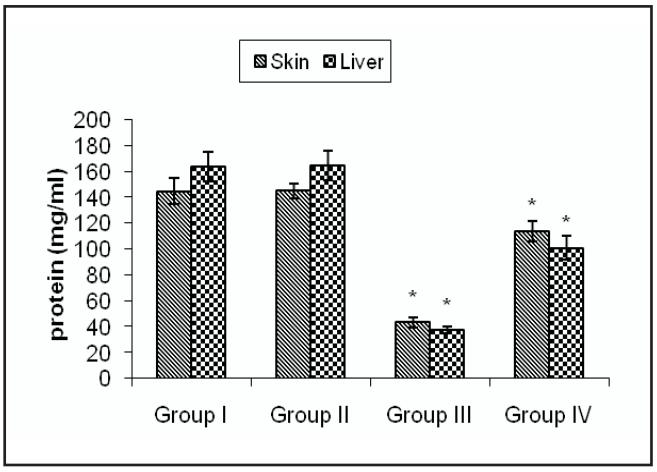


Figure-8. Variation in the protein level in skin during DMBA - induced skin tumorigenesis with or without P.niruri extract administration *p≤0.001



4. Discussion

There has been considerable scientific evidence, epidemiologic and experimental, accumulated in the past two decades indicating that modifications in lifestyle, including diet, can have a major effect on the risk for numerous cancers [23, 24]. Of particular relevance is the consistent cancer-protective effect reported for individuals consuming increased quantities of fruits and vegetables compared with those with low intakes. This cancer inhibitory action by a variety of human nutrients derived from plants as well as of nonnutritive plant-derived constituents (phytochemicals) has been confirmed in different animal tumor models [25, 26] and has led to an increased emphasis on cancer prevention strategies in which these dietary factors are utilized. The present data reveal the modest protective effects of hydro alcoholic extract of phyllanthus niruri on the two stage protocol of the skin papillomagenesis by the observed decreased the tumor burden by 24%, the cumulative number of papillomas by 69%, and the incidence of skin papillomas by 60% in PNE experimental group (Group IV) as compared to the carcinogen control (Group III).

A significant increase in tumor latency (10.52 week) by administration of P.niruri extract in Swiss albino mice initiated by DMBA and later promoted by TPA was also observed. This may be due to the delay in promotion stage as it is found the extract has antioxidant activity and could scavenge oxygen radicals and thus prevent tumour promotion [27]. Even the papilloma size (Table 1) was observed to be significantly smaller in the PNE administered experimental group (Group IV) as compared to the carcinogen treated control (group III).

Oxidative stress arises when the balance between pro-oxidants and antioxidants is shifted towards the pro-oxidants [28]. DMBA is a synthetic polycyclic aromatic hydrocarbon (PAH), which has been used extensively as a prototype carcinogen. The main target sites for the potent carcinogenicity of this agent in rodents are the skin and the mammary gland [29]. It is well known that free radicals generate a cascade, producing lipid peroxidation, protein oxidation, DNA damage and cell death, and contribute to the occurrence of pathological conditions [30, 31]. In the present study the data indicate the protein damage in skin may be due to oxidative stress generated by DMBA/croton oil. In Group IV, oral administration of P.niruri significantly elevated the level of protein as compared to Group III.

During oxidative stress, MDA and/or other aldehydes are formed in biological systems. These can react with amino acids and DNA, and introduce cross linkages between proteins and nucleic acids, resulting in alterations in replication, transcription [32]. and leading to tumor formation. Significant decrease in MDA levels by PNE administration indicates reduced oxidative stress due to increase in GSH and the antioxidant enzyme catalase, thus indicating its protective potential against skin carcinogenesis. Similarly, the findings of the present study also show depletion of GSH with concomitant increase in TBARS level in Group III by DMBA/croton oil alone treatments. In Group IV, oral administration of PNE significantly elevated the level of GSH in skin of mice. Further decrease of GSH with enhanced lipid peroxidation is observed in DMBA/croton oil treated mice suggesting that cells deficient in thiol groups undergo fast lipid peroxidation, as GSH is one of the guarding factors against oxidative stress [33].

Among such numerous biologically active phytochemicals present in *P.niruri*, lupeol, a pentacyclic triterpene, has received much attention due to its wide spectrum of medicinal properties that include strong antioxidant, antimutagenic, anti-inflammatory and antiartheritic effects both in the in vivo and in vitro studies [34,35,36]. It is observed that lupeol treatment to mouse skin resulted in the reduced tumor incidence, lower tumor body burden and a significant delay in the latency period in tumor appearance [36]. It is found that lupeol inhibits DMBA-induced mutagenesis in the skin of Swiss albino mice and exhibit its blocking and suppressing mechanisms of action. Ellagic acid, a component of *P.niruri* that triggers apoptosis, induces G1 inhibition of cancer cell division and prevents destruction of the p53 gene by cancer cells [37]. Phyllanthin, a diaryl butane lignan, isolated from *P.niruri* showed a significant protection against CCl4-induced elevation in transferase levels and significantly increased protein level [38]. ROS formed during DMBA metabolism can diffuse from the site of generation to other targets within the cells or even propagate the injury outside to intact cells. These ROS produce deleterious effects by initiating lipid peroxidation directly or by acting as second messengers for the primary free radicals that initiate lipid peroxidation [39]. Thus, it is essential to provide a suitable range of antioxidants to quench the ROS before they could spread and cause serious damage to mitochondrial DNA, protein and lipids leading to degenerative diseases like cancer [40]. Gallic acid and ellagic acid, present in *P.niruri,* has also been reported to have antioxidant activity and cancer chemopreventive properties [41]. It has been reported earlier that *P.niruri* has potent free radical scavenging activity and could scavenge superoxides and hydroxyl radicals and can inhibit lipid peroxides. Thus, compounds exhibiting antioxidant and/or anti-inflammatory activities are expected to be effective anti-tumour promoting agents [42].

5. Conclusion

It can thus be postulated that the cancer chemopreventive effect of the active bioconstituents of *P.niruri* in the present study could be due to one or more of the following mechanisms: (i) by blocking the carcinogen (DMBA or NOR-1 or DEN) so that it can not pass through the plasma membrane, (ii) by induction of enzymes that detoxify carcinogens (iii) by inhibiting competitively DNA adduct formation (iv) by scavenging reactive.
In general, the beneficial effects of medicinal plants such as *Phyllanthus niruri* may be attributable to one or more phytochemicals, present in them including antioxidants, flavonoids and other substances. Therefore, the result of the present study, suggest that *P.niruri* extract has potential anticarcinogenic and anti-oxidative properties in experimental animals and may be act as a potent chemopreventive agent.

Acknowledgements

Priyanka Sharma gratefully acknowledges for laboratory facilities to Center for Advanced Studies (CAS), Department of Zoology, University of Rajasthan, Jaipur (India). Also my appreciation to Dr. P. K. Goyal for his invaluable guidance and suggestions for carrying out the experiments.

6. References

[1] Agarwal R, Mukhtar H. Cutaneous chemical carcinogenesis in: H. Mukhtar (Ed.). Pharmacology of the Skin. CRC Press, Boca Raton. 1991: pp. 271-387.

[2] Friedrich M, Schwarz M, Furstenberger G. Promotion and cocarcinogenesis. In Arcos JC, Argus M, Woo YT. (eds), Chemical Induction of Cancer, Modulation and Combination Effects. Birkhauser, Boston, MA, 1995: pp. 123-184.

[3] Slaga TJ, Budunova V, Gimenez-Conti I, Aldaz CM. The mouse skin carcinogenesis model. J Invest Dermatol Symp Proc.1996; 1: 151-156.

[4] DiGiovanni J. Multistage carcinogenesis in mouse skin. Pharmacol Ther. 1992; 54: 63-128.

[5] Zhao J, Wang J, Chen Y, Agarwal R. Anti-tumorpromoting activity of a polyphenolic fraction isolates from grape seeds in the mouse skin two stage initiation-promotion protocol and identification of procyanidin B5-30-gallate as the most effective antioxidant constituent. Carcinogenesis. 1999; 20: 1737-1745.

[6] Boone CW, Kelloff GJ, Malone WF. Identification of candidate cancer chemopreventive agents and their evaluation in animal models and human clinical trials: A review. Cancer Res. 1990; 50:2-9.

[7] Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: a review of epidemiological evidence. Nutrition and Cancer. 1992; 18: 1-29.

[8] Wattenberg LW. Inhibition of carcinogenesis by minor dietary constituents. Cancer Research. 1992; 52: 2085S-2091S.

[9] Wang MX, Cheng HW, Li YJ, Meng LM, Mai K. Efficacy of Phyllanthus spp. in treating patients with chronic Hepatitis B. Zhongguo Zhong Yao Za Zhi. 1994 Dec; 19(12): 750-1, 764.

[10] Campos AH, Schor N. Phyllanthus niruri inhibits calcium oxalate endocytosis by renal tubular cells: its role in urolithiasis. Nephron. 1999; 81(4): 393-397.

[11] Santos AR, De Campos RO, Miguel OG, Filho VC, Siani AC, Yunes RA, Calixto JB. Antinociceptive properties of extracts of new species of plants of the genus Phyllanthus (Euphorbiaceae). J Ethnopharmacol. 2000; 72(1/2): 229-238.

[12] Srividya N, Periwal S. Diuretic, hypotensive and hypoglycemic effect of Phyllanthus amarus. Indian J Exp Biol. 1995; 33:861-864.

[13] Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Ray C. Screening of Indian plants for biological activity: I. Indian J Exp Biol. 1968 ;6(4):232-247.

[14] Venkateswaran, P. S., et al. Effects of an extract from Phyllanthus niruri on Hepatitis B and wood chuck hepatitis viruses: in vitro and in vivo studies." Proc. Nat. Acad. Sci. (U.S.A.) 1987; 84(1): 274-278.

[15] Bork PM, Schmitz ML, Weimann C, Kist M, Heinrich M. Nahua Indian Medicinal Plants (Mexico): Inhibitory Activity on NF-κB as an Anti-inflammatory Model and Antibacterial Effects. Phytomedicine 1996; 3: 263-269.

[16] Devi MV. Effect of Phyllanthus niruri on the diuretic activity of punarnava Tablets .J Res Educ Ind Med. 1986; 5: 11-12.

[17] Raphael K R. Anti-mutagenic activity of Phyllanthus amarus (Schum. & Thonn.) in vitro as well as in vivo. Teratog. Carcinog. Mutagen. 2002; 22(4): 285-291.

[18] Ramakrishnan PN, Murugesan R, Palanichamy S, Murugesh N. Oral hypoglycaemic effect of Phyllanthus niruri linn. Ind J Pharmaceut Sci. 1982; 44(1): 10-12.

[19] Moron MA, Depierre JW, Mannervick B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochem Biophys Acta. 1979; 582:67-78.

[20] Ohkhawa H, Ohishi N,Yogi K. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Analyt Biochem. 1979; 95: 351-358.

[21] Aebi H. Catalase: in vitro. In: Colowick SP, Kaplan NO (eds). Method in Enzymology, Academic press, New York 1984; 105: 121-126.

[22] Lowry OH, Rosenbrough NJ, Farr AL, Landall AJ. Protein estimation with folin phenol reagent. J Biol Chem. 1951; 193:265-275

[23] Martinez M, Giovanucci E. Diet and the prevention of cancer. Cancer Metastasis Rev. 1997; 16:357-376

[24] World Cancer Research Fund Food, Nutrition and the Prevention of Cancer: A Global Perspective American Institute for Cancer Research Washington, DC. 1997.

[25] Dragsted L, Srube M, Larsen J. Cancer-protective factors in fruits and vegetables: biochemical and biological background. Pharmacol. Toxicol. 1993; 72(1): 116-135.

[26] Pezzuto J. Plant-derived anticancer agents. Biochem Pharmacol. 1996; 53:121-133.

[27] Joy KL, Kuttan R. Antioxidant activity of selected plant extracts. Amala Research Bulletin. 1995; 15, 68-71.

[28] Prior RL, Cao G. In vivo total antioxidant capacity comparison of different analytical methods. Free Radic Biol Med. 1999; 27: 1173-1181.

[29] Izzotti A, Camoirano C, Cartiglai C, Grubbs CJ, Lubet RA, Kelloff GJ, Flora SD. Patterns of DNA adduct formation in liver and mammary epithelial cells in rats treated with 7,12-Dimethyl-benz(a)anthracene, and relative effects of chemopreventive agents. Cancer Res. 1999; 59: 4285-4290.

[30] Liu J, Wang X, Shigenega MK, Yeo HC, Mori A, Ames BN. Immobilization stress causes oxidative damage to lipid, protein and DNA in the brain of rats. Fed Am Soc Exp Biol J. 1996; 10: 1532-1538.

[31] Olivenza R, Morro MA, Lizasoain I, Lorenzo P, Fernandez AP, Bosca L, Leza JC. Chronic stress induces the expression of inducible nitric oxide synthase in rat brain cortex. J Neurochem. 2000; 74: 745-791.

[32] Perchellet JP, Perchellet M. Antioxidant and multistage carcinogenesis in mouse skin. Free Radic Biol Med. 1989; 7: 377-408.

[33] Lewine WG. Glutathione, lipid peroxidation and regulation of cyt P-450 activity. Life Sci. 1982; 31:779-784.

[34] Moriarity DM, Huang J, Yancey CA, Zhang P, Setzer WN, Lawton RO, Bates RB, Caldera S. Lupeol is the cytotoxic principle in the leaf extract of Dendropanax cf quercetin . Planta Medica. 1998; 64: 370-372.

[35] Nagaraj M, Sunitha S,Varalakshmi P. Effect of lupeol, a pentacyclic triterpene, on the lipid peroxidation and antioxidant status in rat kidney after chronic cadmium exposure. Journal of Applied Toxicology.2000; 20: 413-417.

[36] Saleem M, Afaq F, Adhami V M, Mukhtar H. Lupeol modulates NF-jB and PI3KAkt pathways and inhibits skin cancer in CD-1 mice. Oncogene. 2004; 23: 5203-5214.

[37] Li T M, Chen G W, Su C C, Lin J G, Yeh C C, Cheng KC, Chung JG. Ellagic acid induced p53/p21 expression, G1 arrest and apoptosis in human bladder cancer T24 cells. Anticancer Res. 2005; 25: 971-979.

[38] Syamasundar KV, Singh B, Thakur RS, Husain A, Kiso Y, Hino H. Antihepatotoxic principles of Phyllanthus niruri herbs. Journal of Ethnopharmacology. 1985; 14: 41-44.

[39] Das UN. Aradical approach to cancer. Med Sci Monit. 2002; 8: 7992.

[40] Preston TJ, Abadi A, Wilson L, Singh G. Mitochondrial contributions to cancer cell physiology: potential for drug development. Adv Drug Deliv Rev. 2001; 49: 45-61.

[41] Singh K, Khanna AK, Chander R. Protective effect of ellagic acid on t-butyl hydroperoxide induced lipid peroxidation in isolated rat hepatocytes. Indian J Exp Biol. 1999; 37: 939-943.

[42] Surh YJ. Anti-tumour promoting potential pf selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. Food Chem Toxicol. 2002; 40:1091-1097.