Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.1, No.4, 2011

Protective Effect of *Phyllanthus niruri* on DMBA/Croton Oil Mediated Carcinogenic Response and Oxidative Damage in Accordance to Histopathological Studies in Skin of Mice

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

Radiation & Cancer Biology Laboratory, Department of Zoology, University of Rajasthan, Jaipur-302 004, India

* E-mail of the corresponding author: pkgoyal2002@gmail.com

Abstract

The current study has been designed to unveil the preventive effect of *Phyllanthus niruri* extract (PNE) on two stage skin carcinogenesis and oxidative damage in Swiss albino mice. Single topical application of 7, 12-dimethylbenz (a) anthracene (DMBA), followed by croton oil thrice weekly produced 100% incidence of tumors in carcinogen control animals (Gr. III) by 16 weeks. On the other hand, oral administration of animals with PNE (1 week before of DMBA application & continued until the end of experiment, Gr. IV), significantly reduced the tumor incidence, tumor burden, tumor volume and weight and the number of tumors but prolong the latent period of tumor occurrence, as compared with carcinogen control animals. Furthermore, administration of PNE protected against the losses provoked in levels of glutathione, Vit.C, total proteins and activity of catalase and superoxide dismutase in skin and liver of animals by the application of DMBA/croton oil, concomitantly, the levels of lipid peroxidation were also reduced significantly. *P. niruri* administration profoundly reverted back the pathological changes observed in skin and liver of cancerous animals. From the results, *P. niruri* extract proves to scavenge free radical and found to be a potent chemopreventive agent against chemical induced skin carcinogenesis.

Keywords: carcinogenesis, *Phyllanthus niruri*, cancer chemoprevention, tumor incidence, reactive oxygen species (ROS), antioxidant enzymes

1. Introduction

An increasingly important health problem in the world is the rising incidence of genetic diseases that include age related neurodegenerative diseases, cardiovascular diseases and cancer. Ironically, this is partly due to the increasing longevity of the population, which results from better living and working conditions (Migliore and Coppede` 2002). Even if the etiology of these diseases is not completely understood, but for the sake of improved human health and the quality of life, it will be essential to obtain a better understanding of the key biochemical mechanisms and risk factors for such chronic diseases. Free radicals are found to be involved in both initiation and promotion of multistage carcinogenesis. These highly reactive compounds can act as initiators and/or promoters, cause DNA damage, activate procarcinogens, and alter the cellular antioxidant defense system. Antioxidants, the free radicals scavengers, however, are shown to be anticarcinogens. They function as the inhibitors at both initiation and promotion/transformation stage of carcinogenesis and protect cells against oxidative damage. (Yin sun 1990).

The attractiveness of naturally occurring compounds for cancer chemoprevention has escalated in recent years. An ideal chemopreventive/therapeutic agent would restore normal growth control to preneoplastic or cancerous cells by modulating aberrant signaling pathways and/or inducing apoptosis. It should target the multiple biochemical and physiological pathways involved in tumor development, while minimizing toxicity in normal tissues (Manson *et al.* 2005; Mukhtar and Ahmad 1999a; Mukhtar and Ahmad 1999b; Yance and Sagar 2006).

Among the human diseases treated with medicinal plants cancer is one of the most important genetic disease. Every year, millions of people are diagnosed with cancer, leading to death in a majority of the cases. According to the American Cancer Society (American Cancer Society 2006), deaths arising from cancer constitute 2–3% of the annual deaths recorded worldwide. A large number of chemotherapeutic agents used in cancer treatment have been discovered from natural products. Similarly, several laboratories throughout the world have directed considerable efforts towards discovering new chemopreventive agents from natural products because of their wide biological activities, higher safety right kind of plant material which is therapeutically margins and lesser costs. In this regard the genus *Phyllanthus* includes 500 temperate and tropical species many of which are used medicinally in different countries. *Phyllanthus niruri* L., belonging to family Euphorbiaceae, is a common weed found in both cultivated fields and wastelands in India. It is an annual herb with height varying between 30 and 60 cm. Its roots, leaves, fruits, milky juice, and whole plants are used as medicine (Kirtikar and Basu 1935). Fruits are useful for tubercular ulcers, wounds, sores, scabies and ring worm (Agharkar 1991). The fresh root is believed to be an excellent remedy for jaundice, dropsy and genitourinary infections (Chopra et al. 1956). The infusion of the root and leaves is a good tonic and diuretic when taken cold in repeated doses (Ambasta 1986; Satyavati et al. 1987). In different parts of India, especially, in Chhattisgarh state, it is used as a rich traditional medicine (Caius 1986). *P. niruri* has shown clinical efficacy in viral Hepatitis B for which no effective specific therapy is available (Paranjpe 2001). Fresh juice and powder of dried plant are used most frequently in Ayurvedic preparations (Sastry and Kavathekar 1990). *P. niruri* is still widely used in herbal medicine in South America, remaining the most popular remedy for gallstones and kidney stones throughout the continent (Taylor 2003). In Peruvian herbal medicine, it is also used for hepatitis, urinary infections, and as a diuretic (Devi 1986). There are also reports of a host of other activities of different parts of *P. niruri* and its constituents (Taylor 2003; Bagalkotkar et al. 2006).

Considering the myriads of phytochemicals and therapeutic potential in *Phyllanthus niruri*, the aim of this investigation is to study and evaluate the preventive effect of the plant hydro-alcoholic extract on DMBA/croton oil mediated carcinogenic response and oxidative damage in mammals.

2. Materials and Methods

2.1 Animal care and Handling

The animal care and handling was approved by our institution and was done according to guidelines set by the World Health Organization (WHO), Geneva, Switzerland, and the Indian National Science Academy (INSA), 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, 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 (12x3) hrs. at 40ºC. 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 500 mg/kg body weight.

2.4 Experimental Design

2.4.1 Determination of the effect of P.niruri on DMBA induced carcinogenesis

Group I: Vehicle treated control / Normal (n = 10) - Animals of this group received topical application of acetone (100 µl/ mouse) on the shaven dorsal skin, and double distilled water (equivalent to PNE i.e.100 µl / mouse) was 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 250 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. Two weeks later, croton oil (1% in 100 µl of acetone) was applied as a promoter 3 thrice a 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 (250 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 parameters were taken into consideration:

(i) Cumulative number of papillomas: The total number of papillomas appeared till the termination of the experiment. (ii) Tumor incidence: The number of mice carrying at least one tumor, expressed as a percentage incidence (iii) Tumor yield: The average number of tumors per mouse (iv) Tumor burden: The average number of tumors per tumor bearing mouse (v) Size of tumor & Weight; (vi) Body weight; (vii) 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.

2.4.2 Determination of the effect of P. niruri on enzyme analysis of liver and skin

The animals from all the groups were sacrificed by cervical dislocation 16th week after the commencement of treatments and their liver as well as dorsal skin affected by tumors were quickly excised and washed thoroughly with chilled 0.9% NaCl (pH 7.4). Both the tissues (liver & skin) were then weighed and blotted dry. A 10% tissue homogenate was prepared from the 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 following biochemical parameters. The level of reduced GSH was estimated as an acid soluble non-protein sulfhydryl group by the method of Moron *et al.* (1979). The level of lipid per oxidation was estimated spectrophotometrically by thiobarbituric acid reactive substances (TBARS) method, as described by Ohkhawa *et al.* (1979). Superoxide dismutase activity was assayed by the method of Marklund and Marklund (1974) and the results were expressed as U/mg protein, where U is the unit of enzyme activity defined as the amount of enzyme necessary for inhibiting the 50% auto oxidation of pyrogallol. Auto-oxidation of pyrogallol in Tris-HCL buffer (50 mM, pH-7.5) was measured by the increase in absorbance at 420 nm.

Catalase was assayed on a UV Spectrophotometer at 240 nm by monitoring the decomposition of H_2O_2, as

described by Aebi (1984). The activity of the enzyme was expressed as U/mg of tissue, where U is μ mole of H_2O_2 reduced/min/mg tissue. For the tissue ascorbic acid (Vit.C) determination, the fresh organs were weighed, homogenized in acetate buffer (20mg/ ml) and extracted with cold 4 per cent trichloroacetic acid, centrifuged, and filtered. Ascorbic acid was determined by the method of Roe and Kuether and measured as mg/ml. Protein estimation was determined by the method of Lowry *et al* (1951) using bovine serum albumin as a standard.

2.4.3 Effect of P. niruri on histopathology of Skin

Skin of all the animals were excised out and fixed in Bouin's fixatives for 24-28 hr. and processed by the usual method of paraffin embedding. Sections of 5 μm thickness were taken through microtome, stained with hematoxylin & eosin and were evaluated for pathological changes using binocular light microscope.

2.5 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

3.1 Effect of P. niruri on papilloma formation

Table 1 and 2 show the results of the protective effect of *P. niruri* on DMBA induced skin carcinogenesis. Animals of all groups did not show any significant variation in body weight during the entire experiment and there was no mortality. No noticeable signs of illness e.g. weight loss, diarrhea, alopecia were detected in mice of PNE treated control (Gr. II) group. Similarly no papilloma has been observed in the vehicle treated control (Gr. I) as well as PNE treated control group (Gr. II) whereas the incidence of papilloma was different in DMBA-croton oil treated group (Gr. III 100 %) as compared with the DMBA-croton oil-PNE treated group (Gr. IV 40 %). Cumulative number of papillomas was 62 in carcinogen treated control (Gr. III) which was significantly reduced to 15 in PNE treated experimental group. The tumor burden and yield (3.75 and 1.5 respectively) was considerably reduced in PNE treated experimental group as compared to carcinogen treated control group (6.2). The average tumor weight of the carcinogen treated control was 1.37 gm whereas it was only 0.38 gm for the PNE treated experimental group. The average latent period was observed as 10.66 in PNE treated experimental group which was found to be lesser (i.e.7.93) in carcinogen treated control Group.

3.2 Effect of P. niruri on enzyme analysis of liver and skin

3.2.1 Reduced glutathione

In the levels of GSH 98.04 and 97.13% decrease ($p \leq 0.001$) in liver and skin of animals of carcinogen treated control group (Gr. III), and a 55.12 and 42.38% decrease ($p \leq 0.001$) were observed in both the tissues of animals treated with DMBA/croton oil/PNE as compared with vehicle treated control animals (Gr. I). There was an average 2192.30 and 1907.69% increase ($p \leq 0.001$) in the GSH levels in liver and skin of animals treated with DMBA/croton oil/PNE in relation to both the tissues of carcinogen treated control animals. (Fig. 1)

3.2.2 Lipid peroxidation

The level of LPO in liver and skin of animals treated with DMBA/croton oil (Gr.III) was increased by 390 and 239.39 % ($p \leq 0.001$), whereas animals treated with DMBA/croton oil/PNE (Gr. IV) exhibited only 53.00 and 16.01% increase ($p \leq 0.001$) with respect to vehicle treated control animals. In general, a 69.08 and 65.81 % decrease in the level of LPO was observed in the liver and skin of animals treated with DMBA/croton oil/PNE (Gr. IV) in comparison with the LPO levels in both the tissues of DMBA/croton oil treated animals. (Fig 2)

3.2.3 Superoxide dismutase

In the activity of SOD a decrease of 35.36 and 37.04% ($p \leq 0.001$) was observed in the liver and skin of DMBA/croton oil treated animals (Gr. III), whereas animals treated with DMBA/croton oil/PNE exhibited a 12.32 and 11.17% decrease ($p \leq 0.001$) as compared with the animals of normal (Gr. I). There was an average 35.66 and 41.07% increase ($p \leq 0.001$) in SOD in both the tissues of animals treated with DMBA/croton oil/PNE as compared with the level in DMBA/croton oil-treated animals. (Fig. 3)

3.2.4 Catalase

As compared with vehicle treated control animals (Gr. I), the activity of CAT in liver and skin of carcinogen treated control animals (Gr. III) exhibited a 83.79 and 78.93% decrease ($p \leq 0.001$), whereas 38.70 and 30.57 % decrease ($p \leq 0.001$) was measured in *P.niruri* treated experimental group (Gr. IV). In general, there was nearly 278.15% and 229.61% increase ($p \leq 0.001$), in the activity of CAT in the liver and skin of animals treated with DMBA/croton oil/PNE (Gr. IV) as compared with the activity in the DMBA/croton oil treated animals(Gr.III).(Fig. 4)

3.2.5 Vitamin C

As compared with vehicle treated control animals (Gr. I), the activity of Vit.C exhibited a 30.54% and 37.68% decrease ($p \leq 0.001$) in liver and skin of carcinogen treated control animals (Gr. III), while such decrease was recorded as 7.88 and 10.05 % ($p \leq 0.001$) in *P.niruri* treated experimental group (Gr. IV). In general, there was an 32.62 and 44.35% increase ($p \leq 0.001$), in the activity of CAT in the liver and skin of animals treated with DMBA/croton oil/PNE (Gr. IV) as compared with the activity in the DMBA/croton oil treated animals(Gr.III).(Fig. 5)

3.2.6 Protein

Total proteins level in liver and skin was found to be decreased to 77.04 and 67.26% ($p \leq 0.001$) in carcinogen treated control group (Gr.III), and to 27.37 and 15.04 % ($p \leq 0.001$) in PNE treated experimental group (Gr. IV) as compared with vehicle treated control animals (Gr. I). An average increase ($p \leq 0.001$) of 216.44% and 159.51% in the total proteins level was noted in both the tissues of animals of PNE treated experimental group IV in relation to the protein level of carcinogen treated group.(Fig. 6)

3.3 Effect of P. niruri on histopathology of Skin

The histopathological examination of the skin of carcinogen control animals showed hyperkyratosis (i.e. the thickening of keratinized layer over the epidermis), epidermal hyperplasia, atypical nuclei (enlarged and hyperchromatic), interdermal proliferation, dermal invasion and keratin pearls throughout the epidermis. Some of the cells break through the basement membrane, the process has become invasive. This invasive tumor cells exhibit enlarged nuclei with angulated contours. All of these symptoms were found to be minimal in PNE treated group of animals (Gr. IV) (Fig. 7 A–E).

4. Discussion

Production of reactive oxygen species (ROS) and subsequently oxidative stress cause development of cancer (Guyton and Kensler 1993). The involvement of free radicals in both the initiation as well as the promotion stage, the biochemical events related in each stage and the metabolic alterations associated with cancer development (Boutwell 1974) have been highlighted with the help of two-stage skin carcinogenesis. Plants, vegetables, herbs, and spices used in folk and traditional medicine have been accepted currently as one of the main sources of cancer chemopreventive measures. Therefore, the present study has been designed to evaluate the preventive effect of the hydro-alcoholic extract of *Phyllanthus niruri* on DMBA/croton oil mediated carcinogenic response and oxidative damage. The results show a significant increase in tumor latency by administration of PNE in DMBA initiated and croton oil promoted carcinogenesis. This may be due to the delay in the promotion phase by PNE administration. Besides this, a decrease in tumor weight and size, significant reduction in tumor incidence, tumor burden and cumulative number of papillomas was observed in the group where PNE was given alongwith carcinogen treatment. Even in another study conducted in our laboratory, PNE increased tumor latency and decreased tumor

volume and efficiency of tumor conversion. (Sharma et al. 2009).

Since the compounds exhibiting antioxidant and/or anti-inflammatory activities are expected to be effective anti-tumor promoting agents (Surh 2002), the present study confirms that the phytochemical fraction of *P. niruri*, containing lignans, phyllanthin, hypophylanthin, flavonoids, glycosides and tannin constituents (Murugaiyah et al. 2007 and Bagalkotkar et al. 2006) inhibit the formation of mouse skin tumors. A laboratory study reported that Phyllanthus seems to have the ability to inhibit two of the pro-inflammatory enzymes (COX-2 and iNOS), making it potentially useful in the fight against inflammatory diseases such as cancer (Keimer et al. 2000). Previous studies compared the antidiabetic, hypolipidemic, and antioxidant properties of *Phyllanthus niruri* in normal, insulin-dependent diabetes mellitus (IDDM), and non-insulin-dependent diabetes mellitus (NIDDM) animals through evaluating the effects on carbohydrate and lipid metabolism and antioxidant activities. (Jasmin et al. 2007). Hepatoprotective activity of the phyllanthus extract was also demonstrated *in vivo* by the inhibition of the carbon tetrachloride (CCl_4) – induced formation of lipid peroxides in the liver of rats (Harish and Shivanandappa 2006). Data from these studies and the present study suggest that the antioxidant activity of *P. niruri* is likely to be involved in the reduction of ROS induced by DMBA/croton oil thereby inhibiting tumor development.

Histopathology of the skin and tumors observed at 16^{th} weeks after DMBA/ croton oil treatment showed severe hyperkyratosis (i.e. the thickening of keratinized layer over the epidermis), epidermal hyperplasia, atypical nuclei (enlarged and hyperchromatic), interdermal proliferation, dermal invasion and keratin pearls, suggesting invasive squamous cell carcinoma. On the other hand, intact basal cell layer and dysplastic lesions characterized benign papillomas were observed in DMBA/croton oil/PNE-treated animals (Gr. IV). This direct evidence shows that PNE application inhibits the carcinoma formation and conversion efficiency of papillomas into frank squamous cell carcinoma.

Oxidative stress is associated with the peroxidation of cellular lipids which is determined by measurement of TBA-reactive substances. Lipid peroxidation is increased during the carcinogenic process and malondialdehyde (MDA), a product of lipid peroxidation was observed to be mutagenic and carcinogenic (Apaja 1980 and Basu 1983). The concentration of lipid peroxidation products may reflect the degree of oxidative stress in carcinogenesis. In the present study we evaluated TBA-reactive substances (MDA) to determine the protective activity of PNE from oxidative damage in carcinogenesis. The level of TBA reactive substances in the liver and skin of mice was significantly decreased with PNE administration when compared to the DMBA-induced tumor bearing carcinogen control mice. The decreased level of TBA reactive substances results that PNE can debilitate the pathological conditions of carcinogenesis by inhibition of lipid peroxidation. The phytoconstituents responsible for the inhibition of lipid peroxidation may be due to the presence of flavonoids such as rutin, quercetin, queritrin, etc., present in the plant extract (Bagalkotkar et al. 2006).

Antioxidants are the body's first resource for protection against the diverse free radicals and other oxidative factors (Cross et al. 1987). The antioxidant system comprises various functional components including different antioxidant enzymes, together with the substances that are capable of reducing ROS or preventing their formation. Among them, SOD and CAT enzymes are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative stress (Scott et al. 1991).The non-protein thiol GSH serves as a scavenger of different free radicals and is one of the major defenses against oxidative stress (Halliwell and Gutteridge 1999). Vitamin-C is necessary for the recycling of gluthione and is an effective quencher of reactive oxygen species. The pronounced effect of Vitamin-C in decreasing the incidence and delaying the onset of malignant tumors has already been reported (Pauling 1991). The present study showed the significant reduction ($p < 0.001$) in the activities of antioxidant enzymes (SOD & CAT) and non-enzymatic antioxidant system (GSH & Vit.C) in DMBA treated carcinogen control group (Gr. III) as compared to the PNE treated experimental group (Gr. IV) that could be responsible for increased TBARS levels observed during DMBA-induced oxidative damage. Our result is supported by findings reported previously for liver and skin of mice (Leemol and Kuttan 2001; Chaudhary et al. 2007).The data obtained in experiments proved that the components of *Phyllanthus niruri* extract (PNE) might act as scavengers of reactive oxygen species, and hence could inhibit microsomal peroxidation, membrane destruction and enzyme damage

(Czinner et al. 2001).

Several phytochemicals have been tested in two-stage skin carcinogenesis model, and found to have substantial potential to prevent cancer (Slaga 1984; Singh et al. 2002). Quercetin is one of such component of *P. niruri* extract. Our results and other reports (Herzog et al. 2004; Hansen et al. 1997) suggest quercetin might be one of the active compounds responsible for the anti-carcinogenetic and apoptosis-induction effects of *P. niruri* extract. Ellagic acid is active in antimutagenesis assay and has been shown to inhibit chemically induced cancer in the lung, liver, skin and esophagus of rodents and TPA-induced tumor promotion in mouse skin (Stoner and Mukhtar 1995). Some lipids like ricinoleic acid, linoleic acid, dotriacontanoic acid etc. are components of PNE. These fatty acids may be responsible for the observed anticarcinogenic effects of the present study. Numata et al. (1994) reported that the glyceride fraction from *Coix* seeds containing fatty acids i.e. palmitic, stearic, oleic and linoleic acid inhibit Yoshida sarcoma growth in rats. They suggested that the glyceride components of *Coix* seeds may be metabolized to monolinoleic acid in vivo and this inhibits tumorigenesis. In the present study, perhaps a similar mechanism may be operating in the inhibition of skin carcinogenesis by the PNE.

5. Conclusion

The anticarcinogenic and anti-oxidative activity of hydro-alcoholic extract of *Phyllanthus niruri* (PNE) as observed in our study is due to its stimulatory effect on both enzymatic and non-enzymatic antioxidant systems in the experimental mice. Consequently, the formation of tumors and oxidative damage induced by DMBA/croton oil in the skin of mice is suppressed with the administration of hydro-alcoholic extract of *Phyllanthus niruri* (PNE) due to the reduction in the level of reactive oxygen species (ROS) as indicated by the reduction in the level of TBARS and the induction of recovery and repair process in the liver and skin of mice. The mechanism of action of the extract seems to be (a) suppression of proliferation, (b) suppression of activation of carcinogen, and (c) anti-oxidant activity of the extract. The phytochemicals present in hydro-alcoholic extract of *Phyllanthus niruri* (PNE), responsible for these activities, may be lignans phyllanthin and hypophyllanthin and other components as described above. (Khatoon et al., 2006).

Further studies are needed to elaborate whether some other compounds present in *Phyllanthus niruri* are also responsible for the protective effect against carcinogenesis and oxidative damage and the molecular basis of their mode of action.

6. Acknowledgements and Fundings

Priyanka Sharma gratefully acknowledges for laboratory facilities to Center for Advanced Studies (CAS), Department of Zoology, University of Rajasthan, Jaipur (India). This research received no specific grant from any funding agency in the public, commercial, or not-for- profit sectors.

References

Aebi, H. (1984), “Catalase: in vitro”, In: S.P.Colowick, N.O.Kaplan (eds), Method in Enzymology, *Academic press*, New York, 105, 121-126.

Agharkar, S.P. (1991), “Medicinal Plants of Bombay Presidency”, Scientific Publ., Jodhpur, India, 120–122.

Ambasta, S.P. (1986), “The Useful Plants of India”, Publications and Information Directorate, CSIR, New Delhi, 329.

American Cancer Society, A biotechnology company dedicated to cancer treatment viewed on 25 January 2006. www.cancervax. com/info/index.htm.

Apaja, M. (1980), “Evaluation of toxicity and carcinogenity of malonaldehyde”, An experimental study in Swiss mice, *Thesis Acta Univ Ouluensis* Ser D, 55.

Bagalkotkar, G. et al. (2006), "Phytochemicals from Phyllanthus niruri Linn. and their pharmacological

properties: a review", *The Journal of Pharmacy and Pharmacology* **58**(12), 1559–70.

Basu, A.K., Marnett, J.L. (1983), "Uniquivocal demonstration that malonaldehyde is a mutagen", *Carcinogenesis* **4**, 331–3.

Boutwell, R.K. (1994),"The function and mechanism of promoters of carcinogenesis", CRC *Critical Reviews in Toxicology* **2**, 419–443.

Caius, J.F. (1986), "The Medicinal and Poisonous Plants of India", Scientific Publ, Jodhpur India, 220–223.

Chaudhary, G.. et al. (2007), "Chemopreventive Potential of Aloe vera Against 7,12-Dimethylbenz(a)anthracene Induced Skin Papillomagenesis in Mice", *Integr Cancer Ther* **6**, 405-412.

Chopra, R.N. et al. (1956), "Glossary of Indian Medicinal Plants", CSIR, New Delhi, 191.

Cross, C.E. et al. (1987), "Oxygen radicals and human disease proceedings of a conference", *Ann Intern Med* **107**, 526–545.

Czinner, E. et al. (2001), "The in vitro effect of Helichrysi flos on microsomal lipid peroxidation", *Journal of Ethnopharmacology* **77**, 31–35.

Devi, M.V. (1986), "Effect of Phyllanthus niruri on the diuretic activity of punarnava tablets", *J. Res. Educ. Ind. Med* **5**(1), 11–12.

Guyton, K.Z., Kensler, T.W. (1993), "Oxidative mechanisms in carcinogenesis", *Brit Med Bull* **49**, 523–544.

Halliwell, B., Gutteridge, J.M.C. (1999), "Free radicals in biology and Medicine", 3rd ed. Oxford University Press, Oxford, 237-48.

Hansen, R.K. et al. (1997), "Quercetin inhibits heat shock protein induction but not heat shock factor DNA-binding in human breast carcinoma cells" *Biochem Biophy Res Comm* **239**, 851-856.

Harish, R., Shivanandappa, T. (2006), "Antioxidant activity and hepatoprotective potential of Phyllanthus niruri", *Food Chemistry* **95**(2), 180-185.

Herzog, A. et al. (2004), "Identification of biomarkers for the initiation of apoptosis in human preneoplastic colonocytes by proteome analysis", *Int J Cancer* **109**(2), 220-229.

Jasmin, H. et al. (2007), "Comparative Antidiabetic, Hypolipidemic, and Antioxidant Properties of Phyllanthus niruri in Normal and Diabetic Rats. Pharmaceutical Biology" **45**(7), 569 – 574.

Keimer, A.K. et al. (2000), "Anti-inflammatory potential of Phyllanthus amarus inhibition of iNOS and COX-2 in murine macrophages and in isolated rat Kupffer cells", In: Farnsworth N.R., Wagner, H., Asakama, Y. eds., Supplement II: 3rd International Congress on Phytomedicine, October 11 - 13, 2000, unich: Nature Publishing Group.

Khatoon, et al. (2006), "Comparative pharmacognostic studies of three Phyllanthus species", *Journal of Ethnopharmacology* **104**, 79–86.

Kirtikar, K.R., Basu, B.D. (1935), "In: Indian Medicinal Plants", **3**, 2225.

Leemol, D., Girija, K. (2001), "Effect of Withania somnifera on DMBA induced carcinogenesis", *Journal of Ethnopharmacology* **75**, 165–168.

Lowry, O.H. et al. (1951), "Protein estimation with Folin phenol reagent", *J. Biol. Chem* **193**, 265.

Manson, M.M. et al. (2005), "Innovative agents in cancer prevention", Recent Results in Cancer Research, *Fortschritte der Krebsforschung* **166**, 257–75.

Marklund, S., Marklund, G. (1974), "Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase", *Eur. J. Biochem* **47**, 469–474.

Migliore, L., Coppede, F., (2002), "Genetic and environmental factors in cancer and neurodegenerative diseases", *Mutation Research* **512**, 135–153.

Moron, M.A. et al. (1979), "Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver", *Biochem Biophys Acta* **582**, 67-78.

Mukhtar, H., Ahmad, N. (1999a), "Cancer chemoprevention: future holds in multiple agents", *Toxicology and Applied Pharmacology* **158**, 207–10.

Mukhtar, H., Ahmad, N. (1999b), "Green tea in chemoprevention of cancer", *Toxicol Sci* **5,** 111–7.

Murugaiyah, V., Chan, K.L. (2007), "Determination of four lignans in Phyllanthus niruri L. by a simple high-performance liquid chromatography method with fluorescence detection", *Journal of Chromatography* **1154** (1-2), 198–204.

Numata, M. et al. (1994), "Antitumour components from Chinese herbal medicine", Coix lachryma-jobi, *Planta Medica* **60**, 356–359.

Ohkhawa, H. et al. (1979), "Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction" *Analyt.Biochem* **5**, 351-358.

Paranjpe, P. (2001), "Indian Medicinal Plants: Forgotten Healers", Chaukhamba Sanskrit Pratisthan, Delhi, 48–49.

Pauling, L. (1991), "Effect of ascorbic acid on incidence of spontaneous mammary tumors and UV-light induced tumors in mice", *Am J Clin Natr* **54**, 1252-5.

Roe, J.H., Kuether C.A. (1943), *J. biol. Chem* **147**, 399.

Sastry, T.C.S., Kavathekar, K.R. (1990), "Plants for Reclamation of Wastelands", Publication and Information Directorate, New Delhi.

Satyavati, G.V. et al. (1987), "Medicinal plants of India", Cambridge Printing Works, vol. **2**, ICMR, New Delhi, 166.

Scott, M.D. et al. (1991), "Erythro-cyte defense against hydrogen peroxide: preeminent importance of catalase", *Journal of Laboratory and Clinical Medicine* **118**, 7–16.

Sharma, P. et al. (2009), "Anti-tumor activity of Phyllanthus niruri (a medicinal plant) on chemical-induced skin carcinogenesis in mice", *Asian pacific Journal of cancer Prevention* **10**, 1089-1094

Singh, R.P. et al. (2002), "Silymarin inhibits growth and causes regression of established skin tumors in SENCAR mice via modulation of mitogen-activated protein kinases and induction of apoptosis", *Carcinogenesis* **23**, 499–510.

Slaga, T.J. (1984), "Multistage skin carcinogenesis: a useful model for the study of the chemoprevention of cancer", *Acta Pharmaco Toxicol* (Copenh) **55** (2), 107–24.

Stoner, G.D., Mukhtar, H. (1995), "Polyphenols as cancer chemopreventive agents", *J Cell Biochem* **22**, 169-80.

Sun, Y. (1990), "Free radicals, antioxidant enzymes, and carcinogenesis", *Free Radical Biology and Medicine* **8**(6), 583-599.

Surh, Y.J. (2002), "Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review" *Food Chem. Toxicol* **40**, 1091–1097.

Taylor, L. (2003), "Technical Data Report for Chancap Piedra Stone Breaker (Phyllanthus niruri)", *Herbal Secrets of the Rainforest*, second ed., 17–31.

Yance, D.R. Jr, Sagar, S.M. (2006), "Targeting Angiogenesis with Integrative Cancer Therapies", **5**, 9–29.

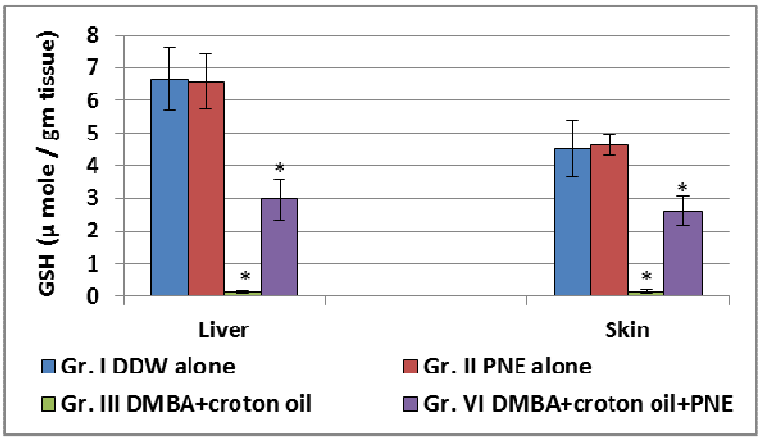

Figure-1 Effect of PNE on reduced glutathione (GSH) content in liver and skin of mice. * ($p \leq 0.001$)

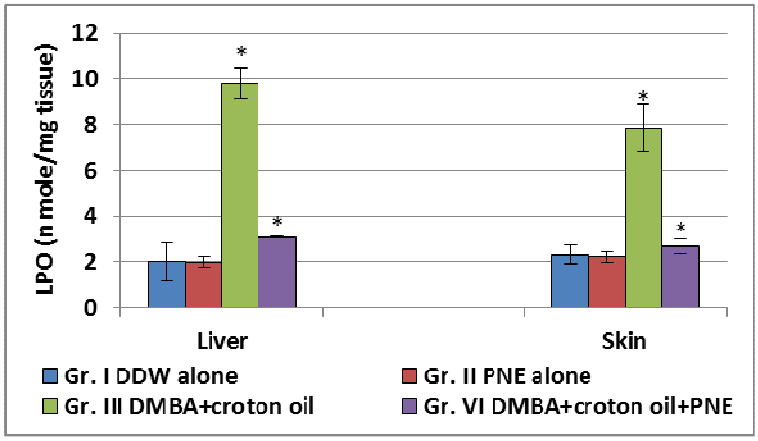

Figure-2 Effect of PNE on lipid peroxidation (LPO) level in liver and skin tissue of mice. * ($p \leq 0.001$)

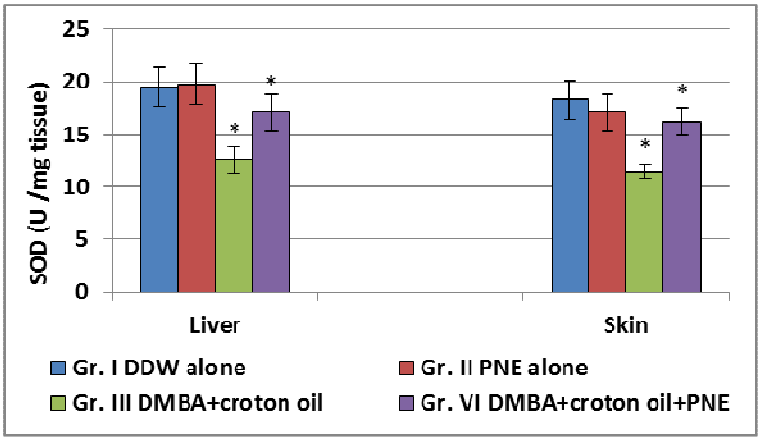

Figure-3 Effect of PNE on the activity of superoxide dismutase (SOD) in liver and skin tissue of mice. * ($p \leq 0.001$)

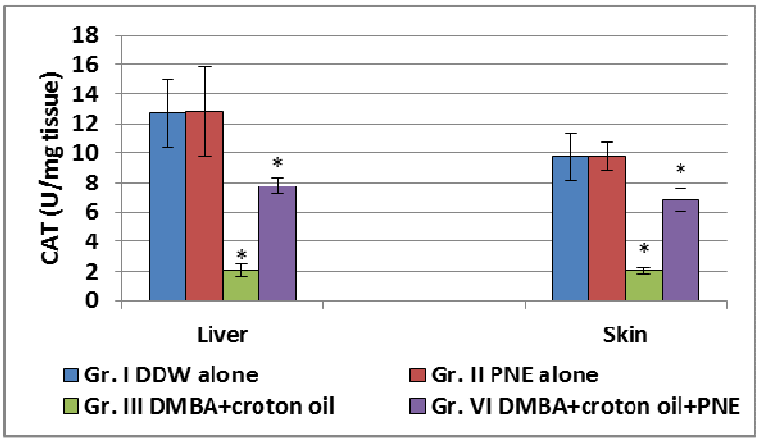

Figure-4 Effect of PNE on the activity of catalase (CAT) in liver and skin tissue of mice. * ($p \leq 0.001$)

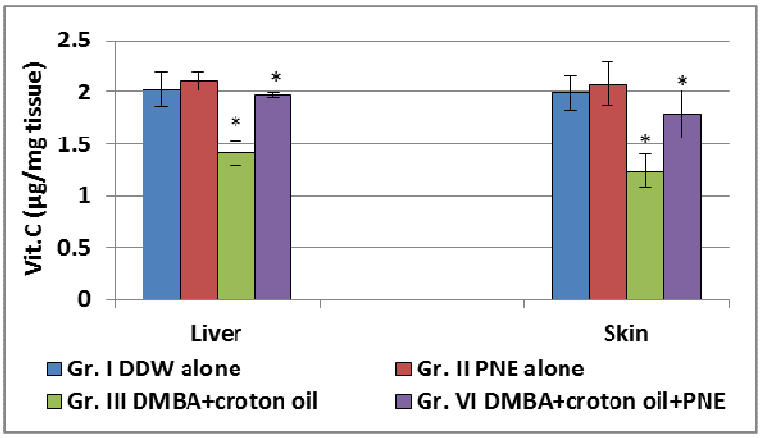

Figure-5 Effect of PNE on vitamin C content in liver and skin tissue of mice. * ($p \leq 0.001$)

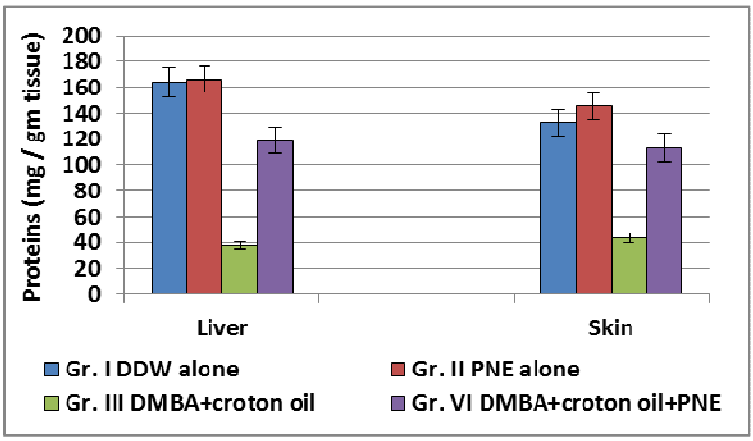

Figure- 6 Effect of PNE on total proteins content in liver and skin tissue of mice. * ($p \leq 0.001$)

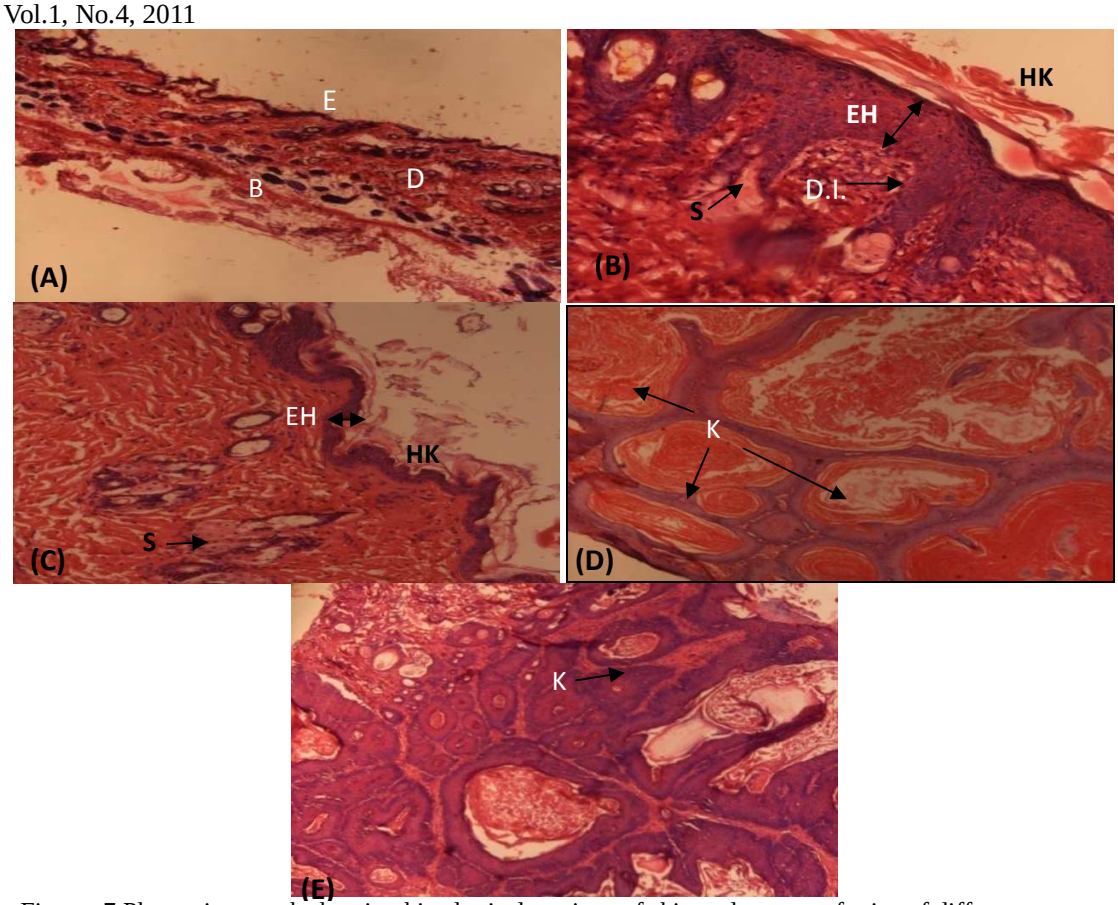

Figure- 7 Photomicrograph showing histological sections of skin and tumors of mice of different groups. (A) Group -I skin, (B) Group- III skin, (C) Group- IV skin, (D) Group-III tumor, (E) Group-IV tumor E- epidermis, D- dermis, B- basal Lamina, HK- hyperkyratosis, E.H- epidermal hyperplasia, D.I.- dermal invasion, S- sebaceous gland, K- keratin pearl

Table 1. Protective effect of *P.niruri* on DMBA induced carcinogenesis in mice

Treatment groups	Body weight (g) Mean ± SE		Tumor size (mm)		Tumor weight (gm)	Average latent period (Weeks)
	Initial	Final	2-5	6-9		
Gr. I Vehicle treated control	25.52±1.72	33.20±1.24	-	-	-	-
Gr. II PNE treated control	26.46±2.18	32.98±1.56	-	-	-	-
Gr. III Carcinogen control	25.60±1.64	30.81±2.29	44	18	1.37	7.93
Gr. IV PNE experimental	25.72±1.58	33.06±1.30	11	4	0.38	10.66

Table 2. Protective effect of *P.niruri* on DMBA induced carcinogenesis in mice

Treatment groups	Cummulative number of Papillomas	Tumor incidence (%)	Tumor yield (average number of tumors/mouse)	Tumor burden (tumors/tumor bearing mouse)
Gr. I Vehicle treated control	-	-	-	-
Gr. II PNE treated control	-	-	-	-
Gr. III Carcinogen control	62	100	6.2	6.2
Gr. IV PNE experimental	15	40	1.5	3.75