Effect of *Phyllanthus Niruri* Leaf Extract on Antioxidant Activity and UV Induced Chromosomal Aberration in *Swiss Albino* Mice

Wasim Raja[a], Sarfaraz Hanfi[a], Sonam Pandey[b], Kriti Tripathi[a], Shabana Hanfi[a] and R. C. Agrawal[b]

[a]Department of Cytogenetics, Genesis Institute of Biomedical Research, Bhopal-462024 Madhya Pradesh, India
[b]Priyamvada Birla Cancer Research Institute, P. O. Birla Vikas, Satna- 485005 Madhya Pradesh, India

Email: **dr.wasimraja@yahoo.com, sarfaraz_hanfi@hotmail.com**

Summary

In the recent study of investigation, the genotoxic effect of leaf extract of *Phyllanthus niruri* has been analysed against UV- induced chromosomal aberration in the bonemarrow cells of mice. Single i.p. administration of *Phyllanthus niruri* leaf extract at three different doses namely 250, 500 and 750 mg/kg b. wt. have provided protection when given 24 hr prior to the exposure of Ultra Violet radiation (UV B, 315 nm). A dose inhibition of chromosomal aberration was observed which was statistically significant ($p<0.05$) as compared to the UV treated group. In another set of experiment, the antioxidant activity of *Phyllanthus niruri* leaf extract using Fenton reaction was also observed and we found a dose dependent inhibition of Thiobarbituric acid reactive substance (TBARS) as compared to positive control. The minimum inhibitory concentration 50% of *P. niruri* and DMSO was found to be 68.76 and 56.98 respectively. Its seems to have a preventive potential against UV induced chromosomal aberrations in the bone marrow cells of the mice and also found antioxidant activity. Therefore, the results suggest a genotoxic and antioxidant potential of *Phyllanthus niruri* leaf extract.

Keywords: Genotoxicity, Ultra Violet, *Phyllanthus niruri*, Bone marrow, Chromosome, Antioxidant activity, Radioprotector.

Introduction

Radiation induced damage to living cells are mediated by the generation of free radicals and related reactive oxygen species (ROS) that damage vital cellular targets such as DNA, membrane lipids and proteins. Naturally occurring antioxidants are also effective radioprotectors due to their ability to scavenge free radicals or neutralize their reactions [1, 2]. These natural radioprotective compounds are of great interest to health management due to their potential applications during radiotherapy in cancer treatment and diagnostic scanning and cleaning operations in nuclear accidents. *Phyllanthus amarus* is a tropical medicinal plant, widely distributed, with many reported beneficial effects. These include antiviral, anti-inflammatory, [3, 4] hypoglycemic, hypocholesteremic, [5] anti bacterial, [6] antifungal [7] and radioprotective [8] activities. Extract of the plant is known to inhibit

gastric carcinogenesis [9] and HIV replication in vitro and ex vivo [10]. The antioxidant activities of the methanolic extracts of *Phyllanthus* were recently demonstrated [11].
The major chemical constituents of *Phyllanthus amarus* are highly heterogenous and complex comprising of lignans like phyllanthin and hypophyllanthin, [12,13] alkaloids, flavonoids and hydrolysable tannins [14-16]. Among these compounds only a few such as gallic acid, ellagic [17] rutin and quercetin [18-21] have been studied extensively for their biological activities. The hydrolysable tannins have been shown to inhibit protein kinases [22] and reviewed by Okuda [23]. The pharmacological actions of polyphenolic compounds may stem mainly from their free radical scavenging and metal chelating properties as well as their effect on cell signaling pathways and gene expression [24].
Most of these species have pharmacological properties e.g. *Phyllanthus niruri* has demonstrated in vitro antibacterial actions against *Staphylococcus, Micrococcus* and *Pasteurella* bacteria as well as *in vitro* and *in vivo* antimalarial properties, which validates other traditional uses of the genus [25]. Extracts of *Phyllanthus* had been used as antiviral source to treat hepatitis B [26-29]. Therefore, we have undertook the preventive effect of *Phyllanthus niruri* using chromosomal aberration assay.

Material and Methods

Animals

The random breed, 6-7 weeks old male *Swiss albino* mice of weight 25 ± 2 gm body were used in the study. These mice were maintained under controlled conditions of temperature (25±2˚C) and light (12 light: 12 dark) and water was given *ad libitum*.

Chemicals:

The cyclophosphamide was purchased from sigma chemical Co., U.S.A. and other chemical were procured locally.

Chromosomal Aberration Assay

For chromosomal assay, three dose of *Phyllanthus niruri leaf extract* i.e. 250, 500 and 750mg/kg b. wt. were administered. *Phyllanthus niruri* leaf extract were dissolved in double distilled water and administered as single dose in 0.2ml per mouse i.p. to 6 animals. Control mice were administered an equal volume of vehicle alone. The positive control group also received a single i. p. injection of 50 mg/kg CP in 0.9% saline. The animals were sacrificed by cervical dislocations and bone marrow cells were harvested. Colchicine (4 mg/kg b. wt.) was administered intraperitoneally 2 hrs. before the harvest of the cells. The slides prepared essentially as per modified method of [10]. Briefly, femur bones were excised and the bone marrow extracted in 0.56% KCL. The harvested cells were incubated at 37°C for 20 mins. and then centrifuged for 10 mins. at 1000 rpm. Cells were fixed in Carnoy's fixative (methanol:acetic acid = 3:1) and bursed opened on clean slides to release chromosome. The slides were stained with 5% Giemsa solution for 15 mins and then put in xylene and mounted with DPX. A total of 100 well spread metaphase plates were scored for chromosomal aberrations at a magnification of 1000 X (100 X 10) for each group. Different types of chromosomal aberration such as chromatid breaks, gaps, centromeric association, etc. were scored and expressed as % of chromosomal aberrations. The statistical significance was determined using Student's 't' test.

***In vitro* Antioxidant activity**

The Fenton reaction was used to generate free radicals in a test system and the free radical scavenging activity was determined by the degradation of deoxyribose as standardized by Elizabeth and Rao (1990) [30]. Fe^{3+}, ascorbate, EDTA, H_2O_2 in the system produced hydroxyl redical which react with deoxyribose and set off a series of reaction that the result is generation of Thiobarbituric acid reactive substance (TBARS). The measurement of TBARS thus gives and index of free radical activity. IC50 values denote the concentration of sample, which is required to scavenge 50% of hydroxyl free radicals. Radicals scavenging by protectors will result in the inhibition of TBARS. This was showed its antioxidant activity.

Results

Data summarized in Table-1 show that *Phyllanthus niruri* leaves extract at dose level 50mg/ml induced significantly prevent different types of chromosomal aberration in bone marrow cells of *Swiss albino* mice. The *phyllanthus niruri* leaves extract administered intraperitoneal route at the dose of 250, 500 and 750 mg/kg b. wt. one hour prior to UV treatment. UV induced different types of chromosomal aberrations in the bone marrow cells of mice. The degree of protection was 18.31, 33.34 and 41.31% respectively. The significant protection was observed with the dose level tested. All kinds of observed aberrations like ring, gap, associations and fragments was found to be protected.

In the positive control group UV induced different types of chromosomal aberrations, *Phyllanthus niruri* leave extract alone did not induced the significant increase in frequency of any of these aberrations at the test dose level and found to be non genotoxic. The tested dose level of *Phyllanthus niruri* leaves extracted against UV induced cell damage.

Table 1: Radioprotective effect of *Phyllanthus niruri* leaf extract on UV induced different types of chromosomal aberrations in bone marrow cells of *Swiss albino* mice.

Sr. No.	Group	Mean ± S.E.	Different types of chromosomal aberration (%)				% of protection
			C.R.	C.F.	C.A.	C.G.	
1.	UV alone	55.96 ± 0.09	-	31.19	24.70	2.70	-
2.	*Phyllanthus niruri* (250mg/kg) + UV	45.71 ± 0.07	-	24.47	18.94	2.10	18.31
3.	*Phyllanthus niruri* (500mg/kg) + UV	37.30 ± 0.97	2.51	19.97	10.03	4.79	33.34
4.	*Phyllanthus niruri* (750mg/kg) + UV	32.84 ± 0.37	1.88	15.34	8.64	6.98	41.31
5.	*Phyllanthus niruri* alone (250mg/kg)	13.0 ± 0.03	-	11.00	2.00	-	-

Antioxidant Activity:

Antioxidant activities of *Phyllanthus niruri* extract using Fenton reaction. The antioxidant activities of *P. niruri* extract scavenge OH^- radical was assessed using the Fenton reaction assay. Extent of hydroxyl radical scavenged was determined by the decrease in intensity of pink coloured, which was determined at 532 nm. The antioxidant activity was compared with DMSO as a positive control. The minimum inhibitory concentration 50% (IC50) of *P. niruri* and DMSO was found to be 68.76 and 56.98 respectively.

Table 2. Antioxidant activity of P. niruri extract using Fenton reaction.

Constrictions (µl)	DMSO	*P. niruri* extract
10	23.34	18.11
20	27.36	21.48
30	35.55	26.89
40	41.78	31.98
50	46.18	38.13
60	53.21	45.55
70	59.74	52.16
80	68.82	56.76
90	77.97	67.94
100	84.31	76.88

Discussion

Naturally occurring antioxidant have been extensively studied for their capacity to protect organism and cells from oxidative damages. Many plant constituents including *Phyllantus niruri* appear to be potent antioxidant property reported by other and our test system. The present data demonstrate that *Phyllantus niruri* leaf extract inhibit the chromosomal aberration induced by UV in mouse bone marrow cells of mice. The antioxidant activity was also observed in vitro test system using Fenton reaction assay and found that *Phyllanthus niruri* leaf extract show free radical scavenge activity. The similar types of studies reported that antiviral, anti-inflammatory, [1,2] hypoglycemic, hypocholesteremic, [5] antibacterial, [6] antifungal [7] and radioprotective [8] activities. Extract of the plant is known to inhibit gastric carcinogenesis [9] and HIV replication in vitro and ex vivo [10]. The antioxidant activities of the methanolic extracts of *Phyllanthus niruri* and *Phyllanthus amarus* were recently demonstrated [11].

The present investigation suggested that possible mechanism may be due to free radical scavenging activites which may be due to presence of in the extract. Hence, it is concluded that *Phyllanthus niruri* extract has an alternative medicine for radioprotection.

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