International Scholars Journals

International Journal of Biochemistry and Biotechnology ISSN 2169-3048 Vol. 8 (9), pp. 001-006, September, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Immunomodulatory activities of methanol extract of the whole aerial part of *Phyllantus niruri* L.

Okorocha Ben Akintola

Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Federal University Oye Ekiti, Oye, Nigeria. E-mail: ben.akintola6@yahoo.com

Accepted 21 March, 2019

In this study, the effect of methanol extract of whole aerial parts of *Phyllantus niruri* on some specific and non-specific immune response was investigated. The effects of *P. niruri* on *in vivo* leucocyte mobilization, delayed type hypersensitivity (DTHR) response, and humoral antibody (HA) response were determined in rats. Acute toxicity profile of *P. niruri* was evaluated in mice. The Agar induced *in vivo* leucocytes mobilization into the rats peritoneal fluid was (P<0.05) increased by *P. niruri* N (200 and 400 mg/kg) in a dose related manner. The total leucocytes count was higher in the extract treated group than the control group. Polymorphonuclear neutrophils (PMNS) were more mobilized than the lymphocytes. *P. niruri* at 100, 200 and 400 mg/kg body weight produced significant (P<0.05) inhibition of DTH response in rat by 30.55, 66.67 and 44.44%, respectively with 200 mg/kg being most significant. The primary and secondary sheep red blood cell antibody titres were significantly elevated when compared with the control group. *P. niruri* administered orally showed no death or signs of acute intoxication at doses up to 5000 mg/kg after 24 h of observation. The result of this study showed that the methanol extract of *P. niruri* whole plant possess immunomodulatory activities and warrant further investigation to determine the specific constituent(s) of the plant responsible for this property.

Key words: *Phyllantus niruri*, leucocyte mobilization, delayed type hypersensitivity (DTHR), humoral antibody titre, immunomodulation.

INTRODUCTION

The immune system is a collection of cells and proteins that works to protect the body from harmful non-self agents such as pathogenic bacteria, viruses and cancers. It utilizes the non-specific (innate) and the specific (adaptive) systems to eliminate these threats. Emerging infectious diseases, bioterrorism, modern day stress are

some of the factors that have made the use of biological response modifiers such as immunomodulators and adaptogens appealing. Recently, there has been a surge in the number of products claimed to boost the immune system, especially herbs. These developments have invariably stimulated a lot of scientific investigations into the veracity of these claims and on the potential benefits of these products to patients. The two arms of the immune response mechanisms can be downregulated or upregulated by immunomodulators. Substances that stimulate the immune system are indicated in the treatment of cancer, immunodeficiency diseases, for generalized immunosuppression following drug treatment, for combination therapy with antibiotics, and as adjuvants for improving vaccines immunogenicity (Nworu et al., 2007, 2010). It has also been suggested that immunomodulatory regimes offer an attractive approach as an adjunct in the control of microbial diseases and in the management of antibiotic resistance (Masihi, 2001). On the other hand, immunosuppressive agents are indicated for conditions associated with hyperimmune responsiveness such as transplanted organ rejection and autoimmune disorders.

Several studies have shown that herbal extracts and supplements possess immunomodulatory properties that could be beneficial if harnessed. One medicinal plant that has been promoted as an immune-boosting agent is *Phyllanthus niruri* Linn. (Family: Euphorbiaceae). *P. niruri* is well-known, versatile herb used medicinally in Africa, Asia, and South America for a variety of ailments (Bagalkotkar et al., 2006). Extracts of this herb have been proven to have therapeutic effects in many animal and clinical studies (Thyagarajan et al., 1988; Calixto et al., 1998; Liu et al., 2001; Xin-Hua et al., 2001; Nworu et al., 2010a, b). Some of the most prominent therapeutic properties include antihepatotoxic (Prakash et al., 1995), antidiabetic (Calixto et al., 1998), anti-HIV (Ogata et al., 1992; Quian-Cutron, 1996), anti-cancer (Rajesh Kumar et al., 2000) and antihepatitis B. (Venkateswaran et al., 1987; Thyagarajan et al., 1992; Shead et al., 1992). It is believed that most of the beneficial effects attributed to the herb are related to its immunomodulatory properties.

P. niruri is a small, erect, annual herb that grows 30 to 50 cm in height (Wikipedia). It is indigenous to the rainforests of the Amazon and other tropical areas throughout the world, including the Bahamas, Southern India and China. *P. niruri* is prevalent in the Amazon and other wet rainforests, growing and spreading freely. *P. niruri* is known in many languages, as Stonebreaker (English), Chanca Piedra (Spanish), and Quebra Pedra (Portuguese). It is a widespread tropical plant commonly found in coastal areas. Many active constituents, responsible for these pharmacological activities of *P. niruri*, have also been identified. Some of the isolated bioactive constituents have been found only in the *Phyllanthus* genus. Generally, biologically active lignans, glycosides, flavonoids, alkaloids, ellagitannins, and phenyl propanoids have been identified in the leaf, stem, and root of the plant (Colombo et al., 2009; Rain Tree Data Base, 2009). In South Eastern Nigeria, *P. niruri* is popularly called “Enyikwonwa” and features in herbal recipes used by herbalist to treat a variety of infections, including claims of effectiveness in the management of HIV/AIDS and hepatitis. Although, it is believed that most of these beneficial effects of *P. niruri* could be related to the immunomodulatory activities, there is scarcity of data on the effects of *P. niruri* on the innate, humoral and cellular immune responses. This motivated the present study in which the effects of the methanol extract of the whole aerial part of *P. niruri* on some specific and non specific immune responses were investigated in murine model.

MATERIALS AND METHODS

Animals

Swiss albino mice (17 to 19 g) and adult rats of Wisterstrain (150 to 220 g) of both sexes obtained from the animal facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka were used in the study. The animals were fed with standard livestock pellets and allowed unrestricted access to drinking water. The rodents were housed under room temperature of 25±2°C and 12 h light/dark cycle.

Antigen

The antigen used in the work is fresh sheep red blood cell (SRBC) obtained from the Animal Farm of the Faculty of Veterinary Medicine, University of Nigeria Nsukka. The sheep red blood cells (SRBCs) was washed three times in a large volume of pyrogen-free sterile normal saline by repeated centrifugation at 2500 rev/s for 10 min on each occasion. The washed SRBC was adjusted to a concentration of approximately 1×10^9 cells/ml and used for both immunization and challenge.

Preparation and extraction of plant

The whole aerial parts of *P. niruri* were collected from the wild in May, 2008 at Imilike, a local community in Nsukka District, Enugu State, Nigeria. The plant was identified and authenticated by Mr. Alfred O. Ozioko, a plant taxonomist of the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State, Nigeria. The whole plant was air dried, and then pulverized. The powdered whole plant (1 kg) was extracted with 3 L of methanol in a soxhlet extractor for 24 h and thereafter concentrated in a rotary evaporator to obtain the solid extract of *P. niruri* (PN; 162.89 g; 16.29% w/w). *P. niruri* was subjected to preliminary phytochemical studies according to the procedures outlined by Harbourne (1984) and Trease and Evans (1996).

Acute toxicity studies

Acute toxicity of *P. niruri* (LD_{50}) was performed in mice using the procedures of Lorke (1983). Briefly, the tests involved two phases. The first phase involved the determination of the toxic range. The

mice were placed in three groups (n=3) and *P. niruri* (10, 100, and 1,000 mg/kg) was administered orally. The treated mice were observed for 24 h for any deaths. The death pattern in the first phase determined the doses used for the second phase according to the Lorke (1983) estimation. In the second phase, four different doses of *P. niruri* were administered (*per os*) as predetermined in the earlier phase of the study. The animals were observed for lethality and signs of acute intoxication for the next 24 h. The LD_{50} was calculated as the geometric mean of the highest non-lethal dose and the least toxic dose.

In vivo leucocytes mobilization rate

The method of Ribeiro et al. (1991) was utilized in the *in vivo* leucocyte mobilization study. One hour after oral administration of the *P. niruri* (100, 200, and 400 mg/kg), each rat in the groups (n=5) received intraperitoneal injections of 0.5 ml of 3% (w/v) agar suspension in normal saline. Four hours later the rats were sacrificed and the peritoneum washed with 5 ml of a 5% solution of EDTA in phosphate buffered saline (PBS). The peritoneal fluid was recovered and total and differential leucocytes counts (TLC and DLC) performed on the perfusates.

Delayed type hypersensitivity response (DTHR)

Delayed type hypersensitivity was induced in rats using SRBC as antigen. Animals were sensitized by subcutaneous injection of 0.2 ml of 1×10^9 cells/ml SRBC (day 0) in the plantar region of the right hind foot paw and challenged on day 5 by subcutaneous injection of the same amount of antigen into the left hind paw. *P. niruri* (100, 200 and 400 mg/kg) were administered 3 days prior to sensitization and continued till the challenge (Shinde et al., 1999; Naved et al., 2005). The oedema produced by antigenic challenge in the left hind paw was taken as the difference in the paw thickness before and 24 h after the challenge. The paw thickness was measured by volume displacement.

Humoral antibody (HA) response

Rats were immunized by an intraperitoneal injection (i.p.) of 0.2 ml of 1×10^9 SRBC/ml (day 0) and challenged by similar i.p. injection of the same amount on day 5. Primary anti body titer was determined on day 5 (before the challenge) and secondary titre on day 10 (Sharma et al., 1996) by the haemagglutination technique (Nelson and Mildenhall, 1967). *P. niruri* (100, 200 and 400 mg/kg) were administered 3 days prior to immunization and continued daily for 5 days after challenge. Blood samples were obtained by retro-orbital puncture in test tubes and allowed to clot. For each sample, a 25 µl serum was obtained after centrifugation and serially diluted two-fold in 96 U-bottom microtitre plates using pyrogen free sterile normal saline. The last well on each row contained sterile normal saline as control. The diluted sera were challenged with 25 µl of 1% (v/v) SRBC in the plates and then incubated at 37°C for 1 h. The highest dilution giving rise to visible haemagglutination was taken as antibody titre. Antibody titre values were expressed in graded manner, the minimum dilution (1/2) being ranked as 1 (calculated as $-\log_2$ of the dilution factor). The mean ranks of different treatment groups were compared for statistical significance.

Statistical analysis

Results of the experiments were analysed using one way analysis of variance (ANOVA, Fischer LSD post hoc test) and expressed as mean ± standard error of mean. Differences between means of treated and control groups were considered significance at $P<0.05$.

RESULTS

Yield and phytochemical analysis

The methanol extraction of 1 kg of *P. niruri* yielded 162.89 g of the drug residue (PN) which represent 16.29% w/w. Phytochemical tests on PN indicated the presence of alkaloids, carbohydrates, resins, tannins, glycosides, reducing sugar, flavonoids, acidic compounds and saponins.

Acute toxicity studies on *P. niruri*

P. niruri administered orally to groups of mice did not cause death or signs of acute intoxication even at doses up to 5000 mg/kg after 24 h of observation.

Effect of *P. niruri* on *in vivo* leucocytes mobilization

Oral administration of *P. niruri* (200 and 400 mg/kg) caused a significant ($P<0.05$) and dose-related increase in *in vivo* leucocyte mobilization in mice by 24.6 and 27%, respectively. The increase was not significant in 100 mg/kg group. Groups of mice treated with *P. niruri* also showed significantly ($P<0.05$) higher number of mobilized neutrophils than the control group (Table 1).

Effect of *P. niruri* on delayed type hypersensitivity response (DTHR) in rats

Short term oral administration of *P. niruri* (100, 200 and 400 mg/kg) caused a significant inhibition of DTHR induced by SRBC in rats up to 30.55, 66.67, and 44.44%, respectively. The highest level of inhibition was observed at 200 mg/kg ($P<0.05$) (Table 2).

Effect of *P. niruri* on primary and secondary antibody production

Haemagglutination titre (HA) showed that oral administration of *P. niruri* in rats produced a dose-dependent increase in primary and secondary antibody production. Administration of *P. niruri* (100, 200, and 400 mg/kg) increased the mean primary anti-SRBC antibody from 3.5 ± 0.29 in the untreated control group of rats to 4.0±0.32, 4.4±0.24 and 4.6±0.24, representing a significant percentage increase of 14.29, 25.71 and 31.43%, respectively. Similarly, the mean secondary anti-SRBC antibody was increased from 4.5±0.29 in negative control to 5.4±0.51, 5.2±0.20 and 7.6±0.40 in groups treated with *P. niruri* (100, 200, and 400 mg/kg), also representing a

Table 1. The effect of PN on *in vivo* leucocyte mobilization in rats.

Treatment	Dose (mg/kg)	TLC (cells mm $^{-3}$)	Increase in leucocytes mobilization (%)	DLC (%)	
				Neutrophils	Lymphocyte
PN	100	652±108	-	49.6±5.0	50.4±5.0
	200	1228±260	24.7*	55.6±6.9*	44.4±6.9
	400	1241±413	26.0*	54.8±8.9*	45.2±8.9
Negative control	Normalsaline	985±180	-	42.0±3.9	58.0±3.9

P< 0.05, n = 5, TLC: Total leucocytes count, DLC: differential leucocytes count, PN: methanol extract of *Phyllanthus niruri*, ±: standard error of mean (SEM).

Table 2. Effects of *Phyllanthus niruri* on delayed type hypersensitivity response (DTHR) in rats

Treatment	Dose (mg/kg)	DTHR Oedema (cm ^{3})	Inhibition (%)
PN	100	0.5±0.09	30.55*
	200	0.2±0.10	66.67*
	400	0.4±0.09	44.44*
Levamisole	25	0.24±0.08	66.67
Negative control	Normal saline	0.72±0.10	-

P<0.05, n=5, PN: Methanol extract of *Phyllanthus niruri*, ±: standard error of mean (SEM).

increase of 20.0, 15.56 and 68.89%, respectively (Figure 1).

DISCUSSION

Strengthening the immune system is a veritable approach that has gained prominence to counter the threats posed by immune destructive diseases like the acquired immune deficiency syndrome (AIDS), emerging virulent and highly pathogenic viruses, and cancers. The innate immunity (which is present at birth and responsible for the provision of first barrier against microorganisms) and the adaptive immunity (which is acquired later in life and acts as second barrier against infection) are the two aspects of immune protection. These two aspects of immune protection could be modified by substances to either suppress or enhance their ability to resist invasion by pathogens (William, 2001).

In this study, the immunomodulatory activities of the whole methanol extract of *P. niruri* on some specific and non-specific immune responses using rodent models was evaluated. Results of the study showed that the administration of the whole methanol extract of *P. niruri* caused a significant (P<0.05) increase in the mobilization of leucocytes into the peritoneum of treated mice in response to injection of agar suspension. The neutrophils count was also correspondingly higher in groups treated with *P. niruri*. Neutrophils are important phagocytic cells involved in innate surveillance and protection against a broad spectrum of pathogens and invaders. They play the main role as effective or killer cells for many types of antigenic challenges especially for infections (Basaran et al., 1997). The primary function of neutrophils in host resistance is their chemotactic migration towards the challenge and the intracellular killing of microorganisms by the formation of oxygen radicals (Badway and Karnovski, 1980).

The results of this study also showed that the delayed type hypersensitivity reaction evoked by SRBCs in mice was inhibited in groups that were treated with the *P. niruri* extract. Macrophages, memory T cells, CD4 and CD8 T cells have been shown to be required for the manifestation of DTHR (Allen, 2013; Sachdeva et al., 2014). It occurs within 24 to 72 h and it has been postulated that Th1 cell is the inducer of DTHR since it secrets interferon gamma (IFNγ), a potent stimulator of macrophages. In DTH reactions, T cells are first recruited into tissues and then activated by antigen presenting cells to produce cytokine that mediate local inflammation (Kalish and Askenase, 1999). It is a major mechanism of defense against various intracellular pathogens, including mycobacteria, fungi and certain parasites, and it occurs in transplant rejection and tumor immunity. This result shows that *P. niruri* can modulate cell mediated adaptive immune response in rodents as shown by the inhibition of DTHR.

In this study, administration of the whole extracts of *P.*

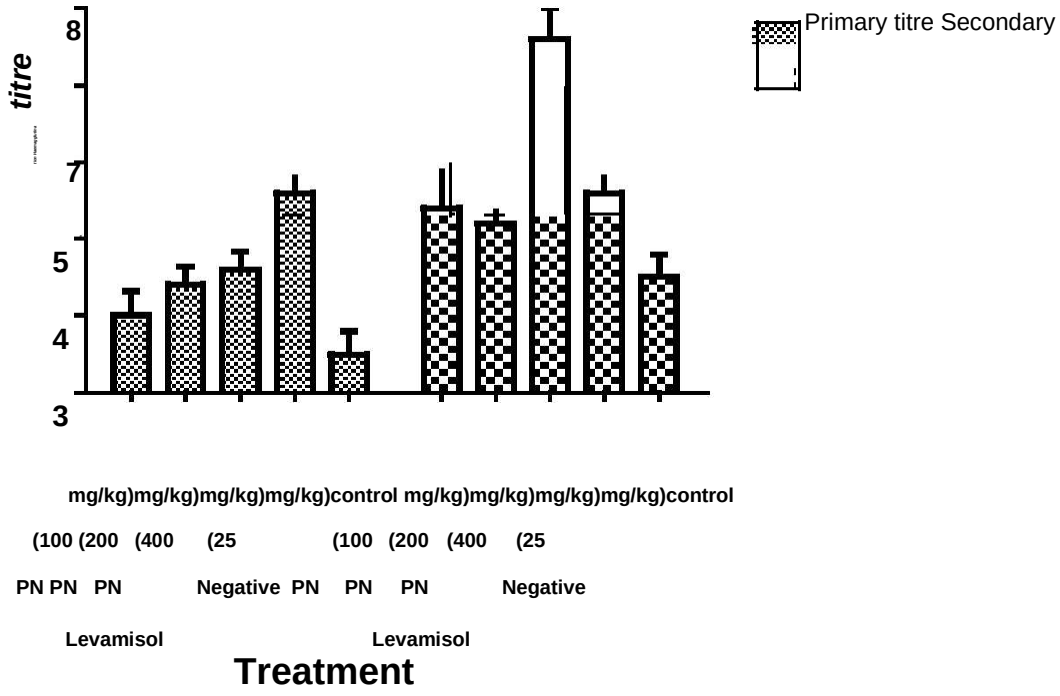

Figure 1. The effects of *Phyllanthus niruri* on primary and secondary antibody responses in rats. P<0.05, n = 5, PN = methanol extract of *Phyllanthus niruri,* haemagglutination titre values are calculated as $\log_2$ of the highest dilution showing visible agglutination and expressed as mean ± standard error of mean (SEM) of 5 animals in each group.
Note: PN: *Phyllanthus niruri*.

niruri caused a significant (P<0.05) elevation of the primary and secondary humoral immune response (antibody) to sheep red cell antigen. The immunoregulatory properties of antibody have been recognized since the earliest passive immunization experiments, and the potential to modulate an immune response by deliberate immunization with antigen bound by antibody has been demonstrated in numerous instances over the decades (Brady et al., 2000; Alber et al., 2001; Antoniou and Watts, 2002; Rafiq et al., 2002). The ability of *P. niruri* to influence humoral response will confer protection to animals or man. This is possible through the utilization of any or combination of the various functions of antibodies synthesized which include agglutination of particulate matter, including bacteria and viruses, opsonization, neutralization of toxins released by bacteria, immobilization of bacteria, activation of complement, mucosal protection, expulsion as a consequence of mast cell degranulation, precipitation of soluble antigen by immune complex formation and antibody dependent cell mediated cytotoxicity. The secondary response are usually far more rapid, high in magnitude, long lived, as witnessed in the results, because during the primary response, some B-lymphocytes in addition to those differentiating into antibody secreting plasma cells, become memory cells which are long lived.

Conclusion

The results of this preliminary investigation have shown that the whole plant methanol extract of *P. niruri* possess immunomodulatory activities and modulates both the innate and adaptive immune components of the immune system. This study postulates that this immunomodulatory activity may be related to the wide ethnomedicinal use of the plant. The specific phytochemical constituent(s) responsible for these immunomodulatory activities was not ascertained in this study and will be the focus of our future investigation.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Alber DIG, Killington A, Stokes A (2001). Solid matrix Antibody –antigen complexes incorporating equine herpesvirus I glycoproteins C and D elicit antiviral immune responses in BALB/C (H-2k(d) and C3H (H-2k CK) mice. Vaccine 19(7-8):895-901.

Allen IC (2013). Delayed-type hypersensitivity models in mice. Methods Mol. Biol.1031:101-107

Antoniou AN, Watts C (2002). Antibody modulation of antigen presentation: positive and negative effects on presentation of the tetanus toxin antigen via the murine B cell isoforms, of FC gamma

R11. Eur. J. Immunol. 32:530-540.

Badway JA, Karnovski ML (1980). Active oxygen species and functions of phagocytic leukocytes. Annu. Rev. Biochem. 49:695-726

Bagalkotkar G, Sagineedu SR, Saad MS, Stanslas J. (2006). Phytochemicals from *Phyllanthus niruri* Linn. and their pharmacological properties: a review. J. Pharm. Pharmacol. 58(12):1559–1570.

Basaran AA, Ceriioghu I, Undeger U, Basaran N (1997). Immunmodulatory activities of some Turkish medicinal plants. Phytother. Res. 11.609–611

Brady LJ, Van Tiburg ML, Aford CE, McArthur WP (2000). Monoclonal antibody mediated modulation of the humoral immune response against mucosally applied streptococcus mutants. Infect. Immun. 68(4):1796-1805.

Calixto JB, Santos AR, Cechinel Filho V, Yunes RA (1998). A review of the plants of the genus Phyllanthus: their chemistry, pharmacology, and therapeutic potential. Med. Res. Rev. 18(4): 225–258.

Colombo R, de L Batista AN, Teles HL, Silva GH, Bomfim GC, Burgos RC, Cavalheiro AJ, da Silva Bolzani V, Silva DH, Pelícia CR, Guimarães FM, Heimberg MC (2009). Validated HPLC method for the standardization of *Phyllanthus niruri* (herb and commercial extracts) using corilagin as a phytochemical marker. Biomed. Chromatogr. 23(6):573-580.

Harbourne JB (1984). Phytochemical Methods: A guide to Modern Technique of Plant Analysis, 2nd ed. London, Chapman and Hall. P 282.

Kalish RS, Askenase PW (1999). Molecular Mechanismsof CD8+T-cell delayed implications mediated hypersensitivity: for allergies, asthma and automimmunity. J. Allergy Clin. Immunol. 103:192-199.

Liu J, Lin H, McIntosh H (2001). Genus Phyllanthus for chronic hepatitis B virus infection: a systematic review. J. Viral Hepat. 8(5):358–366.

Lorke DA (1983). A new approach to practical acute toxicity testing. Arch. Toxicol. 539(4):275-287.

Masihi KN (2001). Fighting infections using Immunomodulating agents. Expert Opin. Biol. Ther. 1(4): 641-653.

Naved T,Siddiqui JI, Ansari SH, Ansar AA and Mukhtar HM (2005). Immunmodulatory activity of *Magnifera Indica* L. fruits (CV. Nee lam) J. Natl. Remedies 5:137-140.

Nelson DA, Mildenhall P (1967). Studies on cytophilic antibodies. The production by mice of macrophage cytophilic antibodies to sheep erythrocyte. Relationship to the development of delayed type hypersensitivity. Aust. J. Exp. Biol. Med. Sci. 45:113-120.

Nworu CS, Akah PA, Okoli CO, Esimone CO, Okoye FBC (2007). The effects of Methanolic seed extract of *Garcinia kola* on some specific and non-specific immune responses in mice. Int. J. Parmacol. 3(4):347-351

Nworu CS, Akah PA, Okoye FBC, Esimone CO (2010a). Aqueous extract of *Phyllanthus niruri* (Euphorbiaceae) enhances the phenotypic and functional maturation of bone marrow-derived dendritic cells and their antigen presentation function. Immunopharmacol. Immunotoxicol. 32(3):393-401

Nworu CS, Akah PA, Okoye FBC, Proksch P, Esimone CO (2010b). The effects of *Phyllanthus niruri* aqueous extract on the activation of murine lymphocytes and bone marrow-derived macrophages. Immunol. Investig. 39(3):245-267.

Ogata T, Higuchi H, Mochida S, Matsumoto H, Kato A, Endo T, Kaji A, Kaji H (1992) . HIV -1 reverse transcriptase inhibitor from *Phyllanthus niruri*. AIDS Res. Hum. Retroviruses 8:1937–1944.

Prakash A, Satyan KS, Wahi SP, Singh RP (1995). Comparativehepatoprotective activity of three *Phyllanthus* Species, *P. urinaria P. niruri and P. simplex*, on carbon tetrachloride-induced liver injury in the rat. Phytother. Res. 9(8):594–596.

Quian-Cutrone J (1996). Niruriside, a new HIV REV/RRE binding inhibitor from *Phyllanthus niruri*. J. Nat. Prod. 59(2): 196–199.

Rafiq KAB, Clynes R (2002). Immune complex mediated Antigen Presentation Induces tumor immunity J. Clin. Investig. 110:71-79

Rain Tree Database (2009). Rain Tree Tropical Plant Database. Available at: http://www.rain-tree.com/chanca.htm#.U38tYdJdU7M Accessed on 9 September, 2009.

Rajesh kumar NV, Kuttan R (2000). Phyllanthus amarus extract administration increases the life span of rats with hepatocellular carcinoma. J. Ethnopharmacol. 73:215–219.

Ribeiro RA, Flores CA, Cunta FQ, Ferraira SH (1991). IL-8 causes in vivo neutrophil migration by a cell dependent mechanism. Immunology 73:472-477.

Sachdeva H, Sehgal R, Kaur S (2014). Asparagus racemosus ameliorates cisplatin induced toxicities and augments its antileishmanial activity by immunomodulation *in vivo*. Parasitol. Int. 63(1):21-30.

Sharma ML, Singh B, Chandan BK, Khajuria A, Kaul A, Bani S, Banerjee SK, Gabbhur SS (1996). Action of some flavonoids on specific and non specific Immune mechanism. Phytomedicine 3:191-195.

Shead A, Vickery K, Pajkos A, Medhurst R, Freiman J, Dixon R, Cossart Y (1992). Effects of Phyllanthus plant extracts on duck hepatitis B virus in vitro and in vivo. Antiviral Res. 18:127–138

Shinde UA, Phadike AS, Nair AM, Mungatiwar AA, Dikshit VS, Saraf MM (1999). Preliminary studies on the immunomodulatory activity of *Cedars deodora* wood oil. Fitoterapia 79:333-339.

Thyagarajan SP, Jayaram S (1992). Natural history of *Phyllanthus amarus* in the treatment of hepatitis B. Indian J. Med. Microbiol. 10:64-84

Thyagarajan SP, Subramanian S, Thirunalasundari T, Venkateswaran PS, Blumberg BS (1988). Effect of *Phyllanthus amarus* on chronic carriers of hepatitis B virus. Lancet 2:764–766.

Trease GE, Evans WC (1996) . Textbook of Pharmacognosy, 14th edn. W.B. Sanders, London. pp 46-47.

Venkateswaran PS, Unander DW, Blumberg BS, Halbherr T, Sharager D, Dahl L, Kraus M, Rissinger C, Simmons HH (1987). Potential antiviral agents for the treatment of hepatitis B virus and retroviruses. Scientia Reports. pp. 300-301.

William JE (2001). Review of antiviral and Immunomodulating properties of plants of the Peruvian rainforest. Alter. Med. Rev. 6:567-579

Xin-Hua W, Chang-Qing L, Xing-Bo G, Lin-Chun FA (2001). Comparative study of *Phyllanthus amarus* compound and interferon in the treatment of chronic viral hepatitis B. Southeast Asian J. Trop. Med. Public Health 32(1):140–142.