



Genotoxicity, cytotoxicity and toxicological evaluation of whole plant extracts of the medicinal plant *Phyllanthus niruri* (Phyllanthaceae)

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ABSTRACT. *Phyllanthus niruri* is a medicinal plant (commonly known as stone breaker) found in the tropics and other parts of the world. It is known for its capacity to block the formation of calcium oxalate crystals and kidney stone formation in urolithiasis. This plant has been used to treat hyperglycemia, hypertension, pain, and mild cases of malaria. We examined the geno-, cyto- and overall toxicity of *P. niruri* whole plant ethanolic extract. The extract was administered as a single dose of 30 or 300 mg/kg to laboratory rats by gavage, accompanied by negative (0.9% saline) and positive (10 mg/mL *N*-ethyl-*N*-nitrosourea) controls that were injected intramuscularly 48 h after extract administration. The ratio of polychromatic (PCE)/normochromatic erythrocytes (NCE) from femur bone marrow was scored for genotoxicity. Cytotoxicity was determined using descending concentrations (0.2-0.0125 g/mL) of the extract incubated with peripheral blood mononuclear cells.

Lactate dehydrogenase release from damaged cells was determined and the CC_{50} calculated. Subchronic administration of the extract at 30 or 300 mg/kg was done for 90 days to determine general toxicity. PCE:NCE (%) for the extract and negative control was 63, compared to 168 (positive control). The CC_{50} was 26.3 $\mu\text{g/mL}$ and hepato-renal toxicity after subchronic extract administration was nil. We conclude that ethanol extract of *P. niruri* is not cytotoxic or genotoxic, and is generally non-toxic on subchronic administration.

Key words: *Phyllanthus niruri*; Micronuclei test; Genotoxicity; Cytotoxicity

INTRODUCTION

The use of plants, plant extracts or plant-derived chemicals to treat diseases is a therapeutic modality that has been explored for centuries. Over 40,000 species of tropical flowering plants are said to possess medicinal properties (Idu et al., 2008) and are currently in use for various medical conditions. The majority of the people of African descent patronize herbal or traditional medicine for their health needs. It is estimated that 70-80% of patients in Africa are treated by traditional healers and herbal practitioners (Diallo et al., 1996; Nyika, 2007). Furthermore, about 40% of all medicines on the market today have been derived directly or indirectly from natural sources; 25% being from plants, 13% from microorganisms and 2% from animals (De Smet, 1997; Pandey, 1998; Shu, 1998; Blumenthal, 1999). However, the safety of these plants has been questioned recently because of fatalities (Ernst and Pittler, 2002). Thus, an assessment of their geno-, cyto- and overall toxicity is imperative.

Phyllanthus niruri, a medicinal plant found in the tropics and other parts of the world, exhibits the ability to block the formation of calcium oxalate crystals (Campos and Schor, 1999; Freitas et al., 2002) and stone formation in urolithiasis (Barros et al., 2003, 2006). Furthermore, it is used to treat hyperglycemia (Raphael et al., 2002), hypertension (Srividya and Periwal, 1995), pain (Miguel et al., 1996), and mild malaria (Tona et al., 2001; Cimanga et al., 2004; Mustofa et al., 2007). *P. niruri* is said to protect against hepatitis B virus (Mehrotra et al., 1990), chemical toxins (Lee et al., 2006; Chatterjee et al., 2006), liver cancer (Rajeshkumar and Kuttan, 2000), and tumorigenesis (Rajeshkumar et al., 2002).

Previous toxicological studies conducted on *P. niruri* have proven that it is toxic to fish and frogs (Kerharo and Adams, 1974). However, an aqueous extract of the plant did not show any toxic effect on cells of the medullar duct of dog kidney (Calixto et al., 1998).

The paucity of evidence from literature (if any) on possible geno- and cytotoxicity, or otherwise of the ethanolic whole plant extract of *P. niruri*, shows that it has not been thoroughly investigated, although its pharmacological efficacy does not appear to be in doubt. Therefore, the aim of this study was to determine the possible geno-, cyto- and overall toxicity of *P. niruri* ethanolic whole extract using *in vivo* and *in vitro* systems.

MATERIAL AND METHODS

The protocol was reviewed and approved by the Institutional Animal Care and Use

Committee of the Noguchi Memorial Institute for Medical Research (NMIMR) according to the Guidelines for Animal Experimentation.

Plant material

Collection and identification of plant material

Whole plants of *P. niruri* used in this study were collected in the months of September and October 2009 from East Legon, in the greater Accra region of Ghana. The plants were sent to the herbarium of the Botany Department of the University of Ghana for authentication and deposition of voucher specimen (No. GC1009). The plant material was then washed thoroughly with water, shade-dried and coarsely milled.

Preparation of ethanolic extracts

Five hundred grams of the dried-milled plant material was extracted twice with 2.5 L ethanol by maceration for 24 h. The mixture was filtered through Whatman filter paper No. 1 and concentrated under vacuum using a rotary evaporator at 55°C. The pooled concentrates were freeze-dried to remove traces of water.

Animal housing conditions

Thirty-eight Sprague-Dawley (S-D) female rats were housed in metal cages with stainless steel tops in the animal care facility of the Center for Scientific Research into Plant Medicine (CSRPM), where room temperature, humidity, and ventilation were controlled according to international standards and were maintained at a 12-h light cycle throughout.

Genotoxicity assays (micronucleus assay)

The mammalian *in vivo* micronucleus test was used for the detection of damage induced by the test substance to the chromosomes or the mitotic apparatus of erythroblasts by analysis of erythrocytes sampled from the bone marrow of rats. Furthermore, the micronucleus test was used to ascertain whether *P. niruri* could cause cytogenetic damage resulting in the formation of micronuclei containing lagging chromosome fragments or whole chromosomes. An increase in the frequency of micronucleated polychromatic erythrocytes in treated animals was therefore an indication of induced chromosome damage.

Twenty female S-D rats were used for this assay. They were grouped as follows: N = 5 for the low dose (LD) group; N = 5 for the high dose (HD) group; N = 5 for negative control, and N = 5 for positive control. Test groups were gavaged with 30 mg/kg body weight (BW) for LD and 300 mg/kg BW for HD. The negative and positive control rats received 500 µL 0.9% saline and 10 mg/mL *N*-ethyl-*N*-nitrosourea, *im*, respectively. Rats were sacrificed after 48 h. The femur bones were removed and the bone marrow gently flushed into tubes containing 0.5 mL fetal calf serum (FCS). The serum was centrifuged at 1000 rpm for 8 min and the sediment resuspended in a drop of the FCS. Thin smears were prepared on slides and air-dried for 24 h. Slides were fixed in absolute methanol for 3 min after which they were stained with a mixture of 12 mL Giemsa, 6 mL

Leishman and 2 mL phosphate buffer, pH 5.8, for 5 min. Afterwards, slides were rinsed with phosphate buffer and air-dried. The slides were then coded for “blind” analyses.

The criteria for scoring followed those of MacGregor et al. (1987) and OECD Guideline 474 (1983). With the oil-immersion objective lens, 1000 polychromatic erythrocytes (PCEs) per animal from coded slides were screened for the presence of micronucleated polychromated erythrocytes (MNPCEs). The number of micronucleated normochromatic erythrocytes (MNNCEs) was registered in a total of 1000 normochromatic erythrocytes (NCEs) per animal. Toxicity to bone marrow was estimated by the relationship between the frequency of PCEs and NCEs. The ratio of PCEs to NCEs was determined in the first 1000 erythrocytes scored per coded slide by two independent microscopists.

Lactate dehydrogenase (LDH) cytotoxicity assay

LDH is released into the surrounding culture medium upon cell damage or lysis during apoptosis and necrosis. LDH activity in any cell culture medium can therefore be used as an indicator of cell membrane integrity and growth, and thus a measure of cytotoxicity.

Peripheral blood mononuclear cells (PBMC) were seeded at a density of 10^5 cells/well in a 120- μ L culture medium into the 96-well culture plate. Descending concentrations (80 μ L) of 0.2, 0.1, 0.05, 0.025, and 0.0125 g/mL *P. niruri* solution prepared under sterile conditions were added in duplicates to the wells containing the PBMCs. The cells were then incubated (37°C, 5% CO₂ and 90% humidity) for 48 h. After centrifugation, the supernatant was analyzed for the level of cytotoxicity. The Cayman cytotoxicity kit (MI, USA) was used according to manufacturer instructions.

Acute toxicity studies

Having determined the LD₅₀ of >3000 mg/kg BW by Sittie A (unpublished data), and showing that *P. niruri* was not acutely toxic at 3000 mg/kg BW, this study proceeded to determine other toxicological potentials of the plant extract.

Subchronic toxicity studies

A total of 18 female S-D rats weighing 150-200 g was used in the subchronic toxicity study. Rats were divided randomly into three groups of six rats per group. Group 1 designated the control, did not receive any plant extract. Groups 2 and 3 received 30 and 300 mg/kg BW, respectively, of the extract daily through the feed for 90 days. Animals were fed *ad libitum*. Body weights of animals were measured at the beginning and at the end of the study (Schlede et al., 1995). All animals were sacrificed on the 91st day. Prior to sacrifice, rats were anesthetized with diethyl ether and blood samples were collected by cardiac puncture. Visible organs (liver, lungs, heart, kidney, and spleen) were macroscopically examined and harvested after blood sampling.

Hematology

Blood for hematological determinations were collected into EDTA anticoagulant

tubes (Sterlin Ltd.). A SYSMEX hematology auto-analyzer (model KX-21N, Japan) was used to determine levels of white (WBC) and red blood cells (RBC), hemoglobin (HBG), hematocrit (HCT), platelet (PLT), and lymphocytes (LYM). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were also determined.

Biochemical assays

Blood samples for serum biochemistry determinations were collected into plain tubes. Samples were centrifuged at 3000 g for 10 min and the following assays determined: renal function tests (urea, creatinine); liver function tests (total protein, albumin, globulin, total bilirubin, direct bilirubin, gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), as well as alkaline phosphatase (ALP) using an auto-analyser (Vitalab Flevor E, Diefen, Netherlands). Urine was collected from rats in each group on days 0 and 91 (before sacrificing). Urine glucose, bilirubin, ketone, blood, nitrite, urobilinogen, specific gravity, pH, protein, and leukocytes were determined using urine reagent strips (URS-10, Teco Diagnostics) for urinalysis.

Histopathology

The harvested organs were examined macroscopically and embedded in paraffin. These were later sectioned into 5- μ m thick slices with a rotary microtome (Leitz microtome, Wetzlar, Germany) and stained with hematoxylin and eosin for examination by light microscopy (Galigher and Kozloff, 1971).

Statistical analysis

The statistical analysis of the data was done using SPSS (Statistical Package for Social Sciences) version 17.0 and the mean \pm SEM was determined. For quantitative data, the Student *t*-test was used to test for significant differences between two variables. Analysis of variance (ANOVA) was used to determine the existence of statistical significance between variables possibly with more than two outcomes. P values ≤ 0.05 were considered to be significant.

RESULTS

Physical observation of toxicity

Signs of toxidromes such as aggression, altered cardiac rate, stupor, excitation, convulsion, rising fur, emesis, etc., were all negative.

Genotoxicity assay

PCE of LD (56.50 ± 18.82) and HD (59.75 ± 8.14) were comparable to the saline negative control (59.17 ± 8.31). NCE levels of test groups and control were in the region of 940 cells. However, NCE of the positive control was 856.20 ± 42.85 . The PCE:NEC ratio (%)

was about 60 for all groups except the positive control that had a ratio of 168. MNNCE was 0 for all groups except the positive control that was 7.00 ± 3.90 . Similarly, MNPCE values were between 1 and 2 for all other groups except the positive control that was 42.00 ± 6.52 . Mononucleated cells for the LD group was 18; HD group = 32; negative saline control = 14; positive control = 72. Finally, binucleated cells were less than 3 in all groups except the positive control that was 69. From the aforementioned, LD and HD of *P. niruri* were non-genotoxic (Table 1, Figure 1).

Table 1. Effect of *Phyllanthus niruri* ethanolic extract on rat bone marrow.

Group	PCE \pm SD	NCE \pm SD	PCE:NCE (%)	MNNCE \pm SD	MNPCE \pm SD	MNPCE distribution	
						α	β
1	59.17 \pm 8.31	940.83 \pm 8.31	62.9	0	1.17 \pm 0.83	14	1
2	143.80 \pm 42.85	856.20 \pm 42.85	168.0	7.00 \pm 3.90	42.00 \pm 6.52	72	69
3	59.17 \pm 7.06	940.50 \pm 7.06	63.3	0	1.67 \pm 0.82	8	2
4	56.50 \pm 18.82	943.50 \pm 18.82	60.0	0	2.08 \pm 1.31	18	3
5	59.75 \pm 8.14	940.25 \pm 8.14	63.5	0	2.75 \pm 1.42	32	1

Group 1 = negative control; group 2 = positive control; group 3 = ethanol control; group 4 = 30 mg/kg; group 5 = 300 mg/kg; PCE = polychromatic erythrocytes; NCE = normochromatic erythrocyte; MNPCE = micronucleated polychromatic erythrocyte; MNNCE = micronucleated normochromatic erythrocyte; α = mononucleated cells; β = binucleated cells; N = 12 slides; SD = standard deviation.

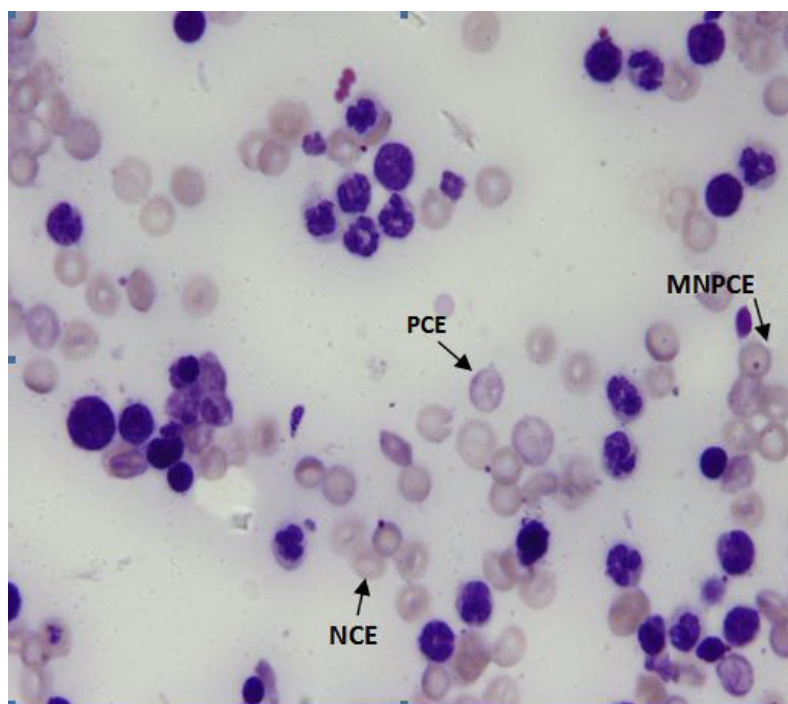


Figure 1. Photomicrograph showing the thin film smear of femur bone marrow aspirate after staining with Giemsa/Leishman stain. Arrows show micronucleated polychromatic erythrocyte (MNPCE), polychromatic erythrocyte (PCE) and normochromatic erythrocyte (NCE) (100X).

Cytotoxicity assay

The effect of *P. niruri* ethanolic extract on the viability of human PBMCs is seen in Figure 2. The results show a dose-dependent increase in % inhibition of cell growth of treated wells compared with the control. The extract at concentrations above 10 $\mu\text{g}/\text{mL}$ recorded % inhibition values of more than 100, compared to the control, and was statistically significant. This was an indication of increased LDH activity in extract treated wells as a result of cell damage, compared to the non-treated wells. From the concentration vs % inhibition curve, the CC_{50} of the extract was estimated as 26.3 $\mu\text{g}/\text{mL}$ (Figure 2).

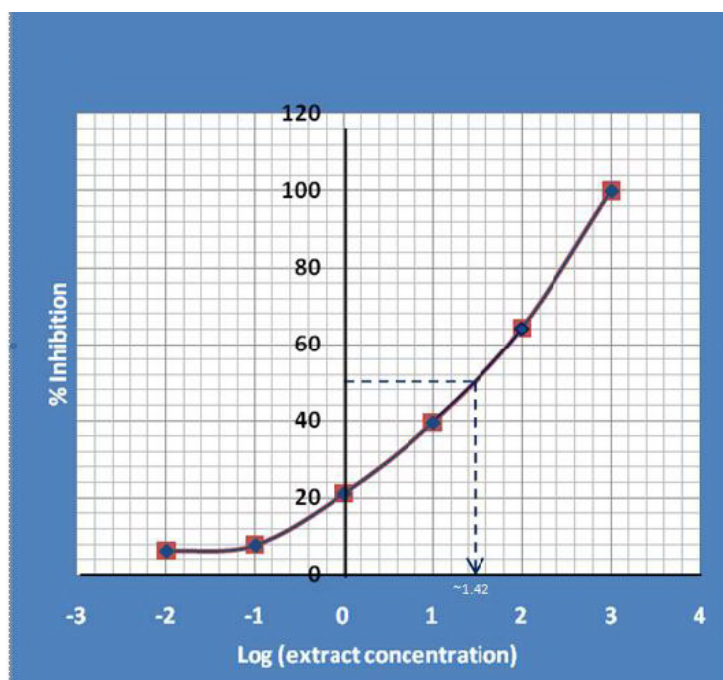


Figure 2. Cytotoxicity of ethanolic extract of *Phyllanthus niruri* whole plant. The graph demonstrates the effect of *P. niruri* whole plant ethanolic extract incubated with human peripheral blood mononuclear cells seeded at 10^5 cells/well. Plant extract in descending concentrations of 0.2-0.0125 g/mL was applied to different wells and incubated for 48 h. Lactate dehydrogenase release was measured as the level of inhibition of cell viability for growth. The CC_{50} , which represents the level of 50% cell growth inhibition, was calculated from the graph as 26.3 $\mu\text{g}/\text{mL}$.

Hematological assays

WBC, RBC, HBG, HCT, MCV, MCH, PLT, platelet distribution width (PDW), mean platelet volume (MPV), and platelet large cell ratio (P-LCR) did not show a statistically significant variation between the control and LD groups. However, in the control group LYM (%) was 82.57 ± 2.43 compared to the LD group, which was 77.58 ± 3.15 . Differences were statistically significant ($P = 0.012$). Furthermore, LYM count was $6.67 \pm 1.18 \times 10^3/\mu\text{L}$ in the

control group, compared to $4.87 \pm 0.98 \times 10^3/\mu\text{L}$ in the LD group. Differences were statistically significant ($P = 0.017$). Standard deviation in red cell distribution width (RDW-SD) was 33.72 ± 2.58 fL in the control group and 38.18 ± 3.36 fL in the LD group with differences being statistically significant ($P = 0.027$). Additionally, coefficient of variation in red cell distribution width (RDW-CV) was $12.4 \pm 1.43\%$ in the control group and $14.57 \pm 1.36\%$ in the LD group ($P = 0.023$) (Table 2). On the contrary, after 90-day continuous administration of 300 mg/kg BW *P. niruri*, there were no statistical differences between the control and HD groups (Table 2). MCH and MCHC differences between the LD and HD groups were statistically significant ($P = 0.004$, $P = 0.034$, respectively), with higher values observed in the latter group. LYM (%) differences between the LD and HD groups were statistically significant ($P = 0.004$). Similarly, HD values were higher than LD values (Table 2).

Table 2. Hematological indices of the control group, low dose group (LD = 30 mg/kg body weight) and high dose group (HD = 300 mg/kg body weight) 90 days after the administration of *Phyllanthus niruri* ethanolic whole plant extract on Sprague-Dawley rats.

Test	Control	30 mg	300 mg	P value
WBC $\times 10^3/\mu\text{L}$	8.08 ± 1.54	6.3 ± 1.41	6.5 ± 1.47	NS
RBC $\times 10^6/\mu\text{L}$	9.02 ± 0.19	9.30 ± 0.36	8.87 ± 0.41	NS
HGB (g/dL)	16.40 ± 0.36	16.72 ± 0.31	16.70 ± 0.62	NS
HCT (%)	55.45 ± 1.58	57.70 ± 1.97	55.72 ± 3.58	NS
MCV (fL)	61.48 ± 1.80	62.05 ± 1.32	62.75 ± 1.48	NS
MCH (pg)	18.18 ± 0.46	18.05 ± 0.44	18.83 ± 0.28	NS
MCHC (g/dL)	29.58 ± 0.26	28.97 ± 0.66	30.02 ± 0.81	NS
PLT $\times 10^3/\mu\text{L}$	870.17 ± 105.40	754.50 ± 259.63	784.83 ± 87.04	NS
LYM (%)	$82.57 \pm 2.43^*$	$77.58 \pm 3.15^*$	84.42 ± 2.32	0.012*
LYM# ($\times 10^3/\mu\text{L}$)	$6.67 \pm 1.18^*$	$4.87 \pm 0.98^*$	5.52 ± 1.31	0.017*
RDW-SD (fL)	$33.72 \pm 2.58^*$	$38.18 \pm 3.36^*$	35.43 ± 2.58	0.027*
RDW-CV (%)	$12.4 \pm 1.43^*$	$14.57 \pm 1.36^*$	12.80 ± 1.41	0.023*
PDW (%)	8.47 ± 0.26	9.20 ± 0.78	8.55 ± 0.48	NS
MPV (fL)	6.88 ± 0.20	7.12 ± 0.23	6.95 ± 0.34	NS
P-LCR (%)	6.52 ± 0.87	8.38 ± 2.44	6.88 ± 1.87	NS

Results of reported as means \pm SD for N = 6. * $P < 0.05$ significantly different from control. WBC = white blood cells; RBC = red blood cells; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelet; LYM% = percent of lymphocytes; LYM# = lymphocyte count; RDW-SD = standard deviation in red cell distribution width; RDW-CV = coefficient of variation in red cell distribution width; PDW = platelet distribution width; MPV = mean platelet volume; P-LCR = platelet large cell ratio.

Biochemical assays

Renal function, assessed by urea and creatinine levels, was as follows: urea (mM): control group = 8.06 ± 0.41 , LD group = 9.76 ± 0.94 and HD group = 9.49 ± 0.79 ; creatinine (μM): control group = 86.78 ± 10.51 , LD group = 91.98 ± 9.43 , and HD group = 94.78 ± 4.59 . Creatinine differences were not statistically significant; however, urea differences were very significant for LD and HD ($P = 0.003$ and $P = 0.013$, respectively,) compared to the control group (Table 3). Liver function tests on the other hand showed marked differences: total bilirubin (μM): control group = 2.42 ± 0.62 , LD group = 0.84 ± 0.51 , and HD group = 1.10 ± 0.75 . The reduction in the LD/HD levels compared to the control was very significant ($P = 0.001$ and $P = 0.007$, respectively). Similarly, for unconjugated bilirubin (indirect bilirubin, μM) a

significant reduction was observed: control group = 1.50 ± 0.59 , LD group = 0.30 ± 0.18 , and HD group = 0.65 ± 0.49 . Differences in LD/HD levels compared to the control group were significant ($P = 0.001$ and $P = 0.021$, respectively). For all liver function enzymes, apart from GGT, LD and HD, levels compared to the control group were statistically significant ($P < 0.05$; Table 3). Significantly different values for GGT was only observed in LD and control groups ($P = 0.002$). With total protein, there was a reduction in the LD group (76.07 ± 3.17 g/L) compared to the control group (83.22 ± 3.93 g/L). Differences were significant ($P = 0.006$). Further significant reductions occurred in the HD group. Similarly, globulin levels decreased in the LD group (33.84 ± 2.02 g/L) compared to the control group (40.98 ± 2.59 g/L). Differences were significant ($P = 0.003$). However, the reductions in albumin in LD and HD groups compared to the control group were not significant (Table 3). Urinalysis results were all negative.

Table 3. Biochemical parameters of the control group, low dose group (LD = 30 mg/kg body weight) and high dose group (HD = 300 mg/kg body weight) 90 days after the administration of *Phyllanthus niruri* ethanolic whole plant extract on Sprague-Dawley rats.

Test	Control	30 mg	300 mg	P value	
Total protein (g/L)	83.22 ± 3.93^{ab}	76.07 ± 3.17^a	69.47 ± 8.79^b	0.006 ^a	0.005 ^b
Albumin (g/L)	42.5 ± 1.41	41.7 ± 1.54	41.93 ± 1.50	NS	NS
Globulin (g/L)	40.98 ± 2.59^a	33.84 ± 2.02^a	42.27 ± 27.42	0.003 ^a	NS
Direct bilirubin (μ M)	0.95 ± 0.05	1.08 ± 0.15	0.78 ± 0.33	NS	NS
Indirect bilirubin (μ M)	1.50 ± 0.59^{ab}	0.30 ± 0.18^a	0.65 ± 0.49^b	0.001 ^a	0.021 ^b
Total bilirubin (μ M)	2.42 ± 0.62^{ab}	0.84 ± 0.51^a	1.10 ± 0.75^b	0.001 ^a	0.007 ^b
GGT (IU/L)	1.46 ± 0.30^a	0.90 ± 0.14^a	1.33 ± 0.21	0.002 ^a	NS
ALT (IU/L)	100.40 ± 24.45^{ab}	21.42 ± 9.84^a	66.73 ± 19.10^b	0.001 ^a	0.023 ^b
AST (IU/L)	180.14 ± 42.91^{ab}	7.46 ± 2.53^a	81.63 ± 32.87^b	0.001 ^a	0.001 ^b
ALP (IU/L)	428.03 ± 47.28^{ab}	352.93 ± 44.80^a	348.48 ± 45.54^b	0.017 ^a	0.021 ^b
Urea (mM)	8.06 ± 0.41^{ab}	9.76 ± 0.94^a	9.49 ± 0.79^b	0.003 ^a	0.013 ^b
Creatinine (μ M)	86.78 ± 10.51	91.98 ± 9.43	94.78 ± 4.59	NS	NS

Results are reported as means \pm SD for N = 6; ^{a,b}Values significantly different, $P < 0.05$. GGT = γ -glutamyltransferase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase.

Histology

Macroscopic examination of the liver, kidney, lungs, spleen, and heart did not reveal any abnormality. Furthermore, histological examination of the various sections did not show any abnormality.

DISCUSSION

Toxicological evaluations of all medicinal plants are important in order to ascertain their safety. Genotoxic substances are potentially known to be mutagenic or carcinogenic. In humans, DNA damage or genotoxicity may be caused by exposure to exogenous agents or substances. On the other hand, endogenous sources of toxins could also result from free-radical action generated by some disease processes. The exposure of cells to genotoxic substances damages chromosomes or components of the mitotic spindle leading to the formation of micronuclei. Therefore, micronuclei may be formed from chromosomal material fragmented during mitosis and not incorporated in daughter nuclei at completion of telophase. Micronuclei are then coated with a nucleus envelope, and their DNA is often transcriptionally active and

undergoes replication (Nusse, 1981; Kramer et al., 1990). The frequency of the presence of micronuclei serves as a measure of *in vitro* or *in vivo* exposure to mutagens and carcinogens. The micronucleus assay has been used in the toxigenetic evaluation of *Ambelania occidentalis*, a plant rich in alkaloids for cancer treatment (Castro et al., 2009) and found to be non-genotoxic. Similarly, the micronucleus test showed that *Memecylon umbellatum* (used for the treatment of gonorrhoea) was not genotoxic (Shetty et al., 2010). *Inula viscosa* (L.) (common name “sticky fleabane”, a perennial weed used for years in folk medicine for antiinflammatory applications) has recently been evaluated for its genotoxic properties. The aqueous leaf extracts of *I. viscosa* induced significant amounts of chromosomal aberrations and micronucleus formation. The results of Askin and Aslanturk (2010) suggest that the *I. viscosa* leaf extract is cytotoxic and genotoxic. Based on the aforementioned reports in literature, it is worthwhile examining the genotoxicity of *P. niruri*, which hitherto had not been documented.

In this study, the cell counts of proportions or percentages of PCE and NCE of the bone marrow cells after LD and HD extract administration were not different from those of the negative control. Furthermore, the estimated ratios of PCE:NEC in bone marrow preparations showed no statistically significant hematopoietic alterations as a result of *P. niruri* ethanolic extract administration. Compared with the *N*-nitroso-*N*-ethylurea-positive control, there was significant increase in DNA damage in femur bone marrow erythrocytes. Therefore, femur bone marrow erythrocytes of S-D rats treated with *P. niruri* did not demonstrate genotoxicity. Similarly, another *Phyllanthus* species, *P. orbicularis* (aqueous extract), showed low cytotoxicity and non-genotoxicity (Sanchez-Lamar et al., 1999). To the best of our knowledge, this is the first study reported on the non-genotoxicity of *P. niruri*.

Recent investigations have revealed that many plants used as food or in traditional medicine have cytotoxic, mutagenic and genotoxic properties (Kassie et al., 1996; Askin and Aslanturk, 2007). In this study, the CC_{50} of the extract was estimated to be 26.3 $\mu\text{g/mL}$. Close results have been obtained, concerning the antiplasmodial activity of stems and leaves of *Phyllanthus reticulatus* against different strains of *Plasmodium falciparum* ($1 \mu\text{g/mL} < IC_{50} < 25 \mu\text{g/mL}$) (Soh et al., 2009). However, Tang et al. (2010) reported much higher levels of cytotoxicity using the methyl tetrazolium salt (MTS) reduction assay. From that study, half-maximal inhibitory concentration (IC_{50}) values of 150-300 mg/mL for aqueous extract and 50-150 mg/mL for methanolic extract of *P. niruri* were obtained. However, the plant extracts did not show any significant cytotoxicity on normal human skin (CCD-1127Sk) and prostate (RWPE-1) cells. In another study on *in vitro* antiplasmodial activity for both the methanolic and aqueous extracts, the IC_{50} values obtained for these extracts against *P. falciparum* ranged from 2.3 to 202.4 $\mu\text{g/mL}$ (Mustofa et al., 2007). Adducing from the aforementioned, *P. niruri* CC_{50} of the ethanolic extract estimated to be 26.3 $\mu\text{g/mL}$ will therefore represent comparatively low or non-cytotoxicity.

In this study, the lymphocytes were reduced significantly with the low dose sub-chronic administration of *P. niruri*. Low lymphocyte count (lymphopenia) can be associated with drugs, especially steroids. Many antineoplastic drugs used to treat cancer produce bone marrow depression and have immunomodulatory effects. The fact that these effects were not observed at the higher dose is difficult to explain. Red blood cell distribution width increases are seen in folic acid, vitamin B6 or iron deficiency anemia. Newly made cells (reticulocytes), B12 and folic acid-deficient cells are larger than iron-deficient cells. The possible reduction in iron may be explained by the presence of phenolics in *P. niruri*, which was phytochemically

determined in this study (Yahaya, 2010). These phenolics have for long been known to impede iron absorption (Kim et al., 2008).

Biochemically, a drastic reduction was observed in most liver function test parameters. Total bilirubin and unconjugated bilirubin were significantly reduced. Similarly, ALT and AST were significantly reduced ($P < 0.001$ and $P < 0.001$, respectively) suggesting some degree of hepatoprotection.

From the data obtained here, we conclude that the *P. niruri* whole plant ethanolic extract is generally non-toxic and that the acute oral administration of *P. niruri* whole plant extract in rats at 300 mg/kg BW does not exhibit genotoxicity.

Conflicts of interest

The authors declare that there is no conflict of interest.

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REFERENCES

- Askin CT and Aslanturk OS (2007). Cytotoxic and genotoxic effects of *Lavandula stoechas* aqueous extracts. *Biologia* 62: 292-296.
- Askin CT and Aslanturk OS (2010). Evaluation of cytotoxicity and genotoxicity of *Inula viscosa* leaf extracts with Allium test. *J. Biomed. Biotechnol.* 2010: 189252.
- Barros ME, Schor N and Boim MA (2003). Effects of an aqueous extract from *Phyllanthus niruri* on calcium oxalate crystallization *in vitro*. *Urol. Res.* 30: 374-379.
- Barros ME, Lima R, Mercuri LP, Matos JR, et al. (2006). Effect of extract of *Phyllanthus niruri* on crystal deposition in experimental urolithiasis. *Urol. Res.* 34: 351-357.
- Blumenthal M (1999). Harvard study estimates consumers spend \$5.1 billion on herbal products? *Herbal Gram.* 45: 68.
- Calixto JB, Santos AR, Cechinel V Filho and Yunes RA (1998). A review of the plants of the genus *Phyllanthus*: their chemistry, pharmacology, and therapeutic potential. *Med. Res. Rev.* 18: 225-258.
- Campos AH and Schor N (1999). *Phyllanthus niruri* inhibits calcium oxalate endocytosis by renal tubular cells: its role in urolithiasis. *Nephron* 81: 393-397.
- Castro LS, Perazzo FF and Maistro EL (2009). Genotoxicity testing of *Ambelania occidentalis* (Apocynaceae) leaf extract *in vivo*. *Genet. Mol. Res.* 8: 440-447.
- Chatterjee M, Sarkar K and Sil PC (2006). Herbal (*Phyllanthus niruri*) protein isolate protects liver from nimesulide induced oxidative stress. *Pathophysiology* 13: 95-102.
- Cimanga RK, Tona L, Luyindula N, Mesia K, et al. (2004). *In vitro* antiplasmodial activity of callus culture extracts and fractions from fresh apical stems of *Phyllanthus niruri* L. (Euphorbiaceae). Part 2. *J. Ethnopharmacol.* 95: 399-404.
- De Smet PA (1997). The role of plant-derived drugs and herbal medicines in healthcare. *Drugs* 54: 801-840.
- Diallo D, Paulsen BS and Hveem B (1996). Production of Traditional Medicine: Preparations Accepted as Medicine in Mali. In: Properties of African Medicinal Plants (Hostettmann K, Chinyanganya F, Millard M and Wolfender J-L, eds.). University of Zimbabwe Publications, Harare, 235-241.
- Ernst E and Pittler MH (2002). Risks associated with herbal medicinal products. *Wien. Med. Wochenschr.* 152: 183-189.
- Freitas AM, Schor N and Boim MA (2002). The effect of *Phyllanthus niruri* on urinary inhibitors of calcium oxalate crystallization and other factors associated with renal stone formation. *BJU Int.* 89: 829-834.
- Galigher AE and Kozloff EN (1971). Essentials of Practical Microtechnique. 2nd edn. Lea and Febiger, Philadelphia.
- Idu M, Timothy O, Omogbai EKI and Amaechina F (2008). Hypotensive effects and acute toxicity property of methanol extract of *Baissea axillaries* Hau. *J. Biol. Sci.* 8: 675-678.
- Kassie F, Parzefall W, Musk S, Johnson I, et al. (1996). Genotoxic effects of crude juices from *Brassica* vegetables and juices and extracts from phytopharmaceutical preparations and spices of cruciferous plants origin in bacterial and

- mammalian cells. *Chem. Biol. Interact.* 102: 1-16.
- Kerharo J and Adams JG (1974). La Pharmacopée Senegalaise Traditionnelle, Plantes Medicinale et Toxiques. Vigot Freres, Paris.
- Kim EY, Ham SK, Shigenaga MK and Han O (2008). Bioactive dietary polyphenolic compounds reduce nonheme iron transport across human intestinal cell monolayers. *J. Nutr.* 138: 1647-1651.
- Kramer J, Schaich-Walch G and Nusse M (1990). DNA synthesis in radiation-induced micronuclei studied by bromodeoxyuridine (BrdUrd) labelling and anti-BrdUrd antibodies. *Mutagenesis* 5: 491-495.
- Lee SK, Li PT, Lau DT, Yung PP, et al. (2006). Phylogeny of medicinal *Phyllanthus* species in China based on nuclear ITS and chloroplast atpB-rbcL sequences and multiplex PCR detection assay analysis. *Planta Med.* 72: 721-726.
- MacGregor JT, Heddle JA, Hite M, Margolin BH, et al. (1987). Guidelines for the conduct of micronucleus assays in mammalian bone marrow erythrocytes. *Mutat. Res.* 189: 103-112.
- Mehrotra R, Rawat S, Kulshreshtha DK, Patnaik GK, et al. (1990). *In vitro* studies on the effect of certain natural products against hepatitis B virus. *Indian J. Med. Res.* 92: 133-138.
- Miguel OG, Calixto JB, Santos AR, Messana I, et al. (1996). Chemical and preliminary analgesic evaluation of geraniin and furosin isolated from *Phyllanthus sellowianus*. *Planta Med.* 62: 146-149.
- Mustofa, Sholikhah EN and Wahyuono S (2007). *In vitro* and *in vivo* antiplasmodial activity and cytotoxicity of extracts of *Phyllanthus niruri* L. herbs traditionally used to treat malaria in Indonesia. *Southeast Asian J. Trop. Med. Public Health* 38: 609-615.
- Nusse M (1981). Cell cycle kinetics of irradiated synchronous and asynchronous tumor cells with DNA distribution analysis and BrdUrd-Hoechst 33258-technique. *Cytometry* 2: 70-79.
- Nyika A (2007). Ethical and regulatory issues surrounding African traditional medicine in the context of HIV/AIDS. *Dev. World Bioeth.* 7: 25-34.
- OECD Guidelines 474 (1983). Available at [www.oecd-ilibrary.org/test-no-474-mammalian-erythrocyte-micronucleus-test_5lmqcr2k7m8w.pdf]. Accessed January 29, 2010.
- Pandey RC (1998). Prospecting for potentially new pharmaceuticals from natural sources. *Med. Res. Rev.* 18: 333-346.
- Rajeshkumar NV and Kuttan R (2000). *Phyllanthus amarus* extract administration increases the life span of rats with hepatocellular carcinoma. *J. Ethnopharmacol.* 73: 215-219.
- Rajeshkumar NV, Joy KL, Kuttan G, Ramsewak RS, et al. (2002). Antitumour and anticarcinogenic activity of *Phyllanthus amarus* extract. *J. Ethnopharmacol.* 81: 17-22.
- Raphael KR, Ajith TA, Joseph S and Kuttan R (2002). Anti-mutagenic activity of *Phyllanthus amarus* Schum & Thonn *in vitro* as well as *in vivo*. *Teratog. Carcinog. Mutagen.* 22: 285-291.
- Sanchez-Lamar A, Fiore M, Cundari E, Ricordy R, et al. (1999). *Phyllanthus orbicularis* aqueous extract: cytotoxic, genotoxic, and antimutagenic effects in the CHO cell line. *Toxicol. Appl. Pharmacol.* 161: 231-239.
- Schlede E, Mischke U, Diener W and Kayser D (1995). The international validation study of the acute toxic class method (oral). *Arch. Toxicol.* 69: 659-670.
- Shetty P, D'Souza UP and Prasanna SK (2010). Genotoxic studies of *Memecylon umbellatum* leaves. *Int. J. Pharm. Health Sci.* 1: 45-49.
- Shu YZ (1998). Recent natural products based drug development: a pharmaceutical industry perspective. *J. Nat. Prod.* 61: 1053-1071.
- Soh PN, Banzouzi JT, Mangombo H, Lusakibanza M, et al. (2009). Antiplasmodial activity of various parts of *Phyllanthus niruri* according to its geographical distribution. *Afr. J. Pharmacy Pharmacol.* 3: 598-601.
- Srividya N and Periwal S (1995). Diuretic, hypotensive and hypoglycaemic effect of *Phyllanthus amarus*. *Indian J. Exp. Biol.* 33: 861-864.
- Tang YQ, Jaganath IB and Sekaran SD (2010). *Phyllanthus* spp. induces selective growth inhibition of PC-3 and MeWo human cancer cells through modulation of cell cycle and induction of apoptosis. *PLoS One* 5: e12644.
- Tona L, Mesia K, Ngimbi NP, Chrimwami B, et al. (2001). *In-vivo* antimalarial activity of *Cassia occidentalis*, *Morinda morindoides* and *Phyllanthus niruri*. *Ann. Trop. Med. Parasitol.* 95: 47-57.
- Yahaya ES (2010). Toxicological Effects of *Phyllanthus niruri* Ethanolic Extract in Sprague-Dawley Rats. Master's thesis, Department of Pharmacology, University of Ghana, Accra.