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Influence of *Phyllanthus Amarus* on Biochemical and Haematological Status in Rats with Ibuprofen-Induced Nephrotoxicity

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Abstract

This present study was designed to evaluate the influence of 70% alcohol extract of *Phyllanthus amarus* leaf (AEPA) on the biochemical and haematological status in rats with ibuprofen-induced nephrotoxicity. Twenty-four albino Wistar rats (150-190 g) of both sexes were randomly grouped into six of four rats per group. Group I served as the control and was administered distilled water. Groups II-VI were induced by oral gavage with 200 mg/kg b.wt. of ibuprofen single dose. On the third day of the experiment, group III was treated with 0.097 mg/kg b.wt. of termisartan drug while groups IV, V and VI were treated with 50, 100 and 200 mg/kg b.wt./day of AEPA respectively for 28 days. Then, biochemical and haematological parameters were measured while histological study was carried out on kidney sample. No significant ($p>0.05$) change in body weight of animals throughout the experimental period. Treatment of rats with 50-200 mg/kg b.wt of *P. amarus* leaf extract and termisartan drug significantly ($p<0.05$) reduced the levels of creatinine, AST, ALP. RBC, HB, HCT and PLT and significantly ($p<0.05$) increased ALT, total protein and albumin levels, monocyte and neutrophil counts compared to the ibuprofen-induced rats group. No significant ($p>0.05$) alterations were observed in WBC, MCV, MCH, MCHC and lymphocytes. The induced morphological architecture alterations in the kidney by ibuprofen were reversed with increased doses of *P. amarus*. Therefore, AEPA has selective ameliorative influence on the biochemical and haematological parameters in ibuprofen-induced nephrotoxic rats.

Keywords: Phyllanthus amarus; Biochemical; Heamatological; Ibuprofen; Nephrotoxicity.

1. Introduction

Herbal plants have been used to treat various ailments and diseases by rural and tribal communities since ancient times. Nowadays, the use of medicinal plants has not been restricted to the rural communities alone, the urban and cosmopolitan communities, particularly in the third world countries, have been utilizing herbal products to treat various human diseases due to their easy access and low cost, compared with advanced Western synthetic medicines [1-3]. The World Health Organization (WHO) has reported that about 80% of population in the developing countries such as Nigeria, depends on herbal medicines or phytomedicines for the treatment of a number of diseases [4, 5]. Over fifty percent (50%) of all modern chemical drugs are of natural plant product origin, and is essential in drug development programs of the pharmaceutical industry [6]. This has led researchers, in recent times, to focus on the exploration of more medicinal flora for novel alternative drug resources [2, 7].

Ibuprofen, a propionic acid derivative, is an example of the non-steroidal anti-inflammatory drugs (NSAIDs), which are among the most frequently prescribed medications worldwide [8, 9]. Ibuprofen is one of the most commonly used NSAIDs for the relief of fever, pains and inflammatory conditions. Although, NSAIDs are generally considered to have high safety profiles, the frequent and widespread use of ibuprofen and other NSAIDs is likely to increase the prevalence of their adverse effects. Ibuprofen and other NSAIDs are commonly associated with gastrointestinal (GI) toxicity [10, 11]. In addition, NSAIDs have been shown in previous studies to alter renal function [12, 13]. However, most of such reports are on high dose levels of the agents (> clinical doses) and existing data on ibuprofen-mediated renal toxicity in relation to duration of exposure is not exhaustive. NSAIDs are also

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known to have antiplatelet activities [14]. However, the antiplatelet effects of ibuprofen in relation to dose and duration of exposure has not been fully established.

Phyllanthus is the largest genus in the plant family Phyllanthaceae. It has 11 sub-genus with over 700 known species, are cosmopolitan and are mainly distributed in the tropics and subtropics [15]. *Phyllanthus* is with a long history of use for the treatment of liver, kidney and bladder problems, diabetes and intestinal parasites [16]. The *Phyllanthus amarus* species is commonly found in tropical and subtropical countries like India, Cuba, China, Philippines, Sierra Lone, Congo Brazzaville and Nigeria [17]. In Nigeria, the plant is commonly called *dobisowo* or *ehin olobe* or *ehin olubi sowo* in Yoruba, Southwest and *ngwu* by in Igbo, Southeast and *buchi oro* by the Asaba people, South south [18]. It is known in Hindi, India as *Bhuyiavla* and *Jangli amla* where is commonly used in the Indian Ayurvedic system of medicine in treatment of stomach, genitourinary system, liver, kidney and spleen problems. In English, it is known with different names like Carry me seed, Child pick-a-back, Gale of wind, Gulf leaf flower, Hurricane weed, Shatterstone and Stone breaker while it is *Poudre de plomb* in French (Ivory coast), *Weisse Blattblume* (German) and *Yerba magica* in Spanish (Cuba) [19]. *P. amarus* is a branching annual glabrous herb that is 30 - 60 cm high and slender, bearing leaf-branchlets and distichous leaves with subsessile, elliptic-oblong, obtuse, rounded base [20]. Traditionally, the plant has been reportedly used to treat diarrhoea, dysentery, dropsy, intermittent fevers, jaundice, urinogenital disorders, wounds and scabies [21-23]. It is also found to be useful in treatment of kidney problems, urinary bladder disturbances, gonorrhea, pain, diabetes, chronic dysentery, appendix, inflammation and prostate problems [23, 24]. In Nigeria, the leaves and whole plant are boiled in water as decoction or infused in alcohol and used for stomachache, malaria and diabetes treatment [25-27].

Alkaloids, tannins, lignans, flavonoids, triterpenes, sterols and volatile oil are the secondary metabolites reportedly present in *P. amarus* while many phytochemical compounds have been isolated from the plant. Some of these isolated compounds include phyllanthin, hypophyllanthin, lintetralin, isolintetralin, (3-(3,4-dimethoxy-benzyl)-4-(7-methoxybenzo[1,3]dioxol-5-yl-methyl)-dihydrofuran-2-one, linalool and phytol [28-32]. Several pharmacological activities have been reported, these include anti-diabetic [33], antimicrobial [34], anti-inflammatory [35], antioxidant [23], antiviral [36, 37], anticancer [38], anti-infertility [39], anticonvulsant [40], nephroprotective and cardioprotective [41], hepaprotective [42, 43] and haematological property [44, 45]. The plant leaf extract has also shown anti-diarrhoeal, gastro-protective and anti-ulcer [46], hypoglycemic and hypocholesterolemic [47], immunosuppressive [48], antinociceptive [49] and spasmolytic [50] activities.

Though, *P. amarus* has been reported to exhibit many pharmacological potentials, the toxic nature of the plant has also been reported by few authors. Histological studies of aqueous extracts of the plant on the kidney has showed some varying degree of distortion and disruption in microanatomy of the kidney including interstitial oedema, inflammatory cells and degrees of tubular necrosis [51, 52];. At the same time, ibuprofen is an over the counter pain relieving drug that is subject to abuse. This drug has also been reported to have the potential to damage the kidney of user when not carefully used. Therefore, this study was designed to evaluate the influence of *Phyllanthus amarus* leaf extract on biochemical and haematological status of rats in ibuprofen-induced nephrotoxicity.

2. Materials and Methods

2.1. Drugs and Chemicals

Ibuprofen 400 mg was obtained from Fidson Pharmaceuticals Limited, Nigeria while telmisartan USP 40 mg was manufactured by MSN Laboratories Private Limited, marketed and distributed by Phillips Pharmaceuticals Nigeria Limited, Nigeria. Reagents used were of analytical grade mainly obtained from renowned manufacturers like British Drug House (BDH) Ltd., Poole, England and Sigma-Aldrich Laborchemkalien GmbH, Seelze, Germany. Diagnostic reagent kits used for the biochemical assays were products of Roches Diagnostics USA.

2.2. Collection of Plant Material and Preparation of Extract

Fresh areal parts of *Phyllanthus amarus* were collected from Sango-Otta, Ogun State, Southwest Nigeria. Plant sample was identified by Mr. Adeleke of the Department of Pharmacognosy, Faculty of Pharmacy, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria. The plant was thoroughly cleaned with running tap water and air-dried on the laboratory bench under normal atmospheric temperature for 7 days. Dried leaves were separated from stalks and ground to fine powder using porcelain mortal and pestle. Alcoholic extract was prepared by soaking 100 g dried powder in 2 L 70% ethanol for 72 h and shaking intermittently. This was filtered with a muslin cloth and then Whatman No.1 filter paper and filtrate was concentrated and evaporated at 40^{o}C under reduced pressure using rotary evaporator to obtain the extract (AEPA).

2.3. Animal

Twenty four adult male and female wistar rats (180-230 g) were obtained from the Animal House, Lagos State University College of Medicine (LASUCOM), Ikeja, Lagos, Nigeria. The animals were randomly distributed into six groups of four rats per cage, housed in standard aluminum sheet cages and allowed to acclimatize for 14 days in well ventilated animal research laboratory room of the College, at room temperature (30°C ± 2°), relative humidity (70-78%) and natural lighting condition (12 h photo period). The animals were fed with standard rodent pellets and allowed free access to water *ad libitum.* The animals were handled in accordance with the international, national and institutional guidelines for Care and Use of laboratory Animals as promulgated by the Canadian Council on Animal Care. All experimental protocols described were approved by the Ethical Committee of the College.

2.4. Experimental Design

After the acclimatization period, the rats received treatments as shown below.

Group I: Normal control rats, received distilled water alone

Group II: Nephrotoxic control rats, received a single dose of 200 mg/kg b.wt. ibuprofen only

Group III: Nephrotoxic rats treated with 0.097 mg/kg b.wt. termisartan drug

Group IV: Nephrotoxic rats treated with 50 mg/kg b.wt. AEPA

Group V: Nephrotoxic rats treated with 100 mg/kg b.wt. AEPA

Group VI: Nephrotoxic rats treated with 200 mg/kg b.wt. AEPA

Twenty four male and female adult wistar rats weighing 180-230 g were distributed randomly into six groups (I-VI) of four rats per group. Group I served as control and received distilled water throughout the duration of the experiment. Groups II-VI were administered single dose of 200 mg/kg b. wt ibuprofen for 72 h to induce nephrotoxicity in the rats. Thereafter, group II was given distilled water, group III was treated with single dose of 0.097 mg/kg b.wt./day termisartan while groups IV, V and VI were treated with single dose of 50, 100 and 200 mg/kg b.wt./day AEPA respectively for a consecutive period of 28 days. All administrations of drugs and extract to the rats were carried out by oral gavage.

2.5. Collection of Samples

At the end of the administration, the animals were starved overnight and then anaesthetized with ketamine. Blood samples were collected by heart puncture separately into heparin and EDTA bottles for biochemical and hematological analyses respectively. The blood sample in heparin bottles was immediately centrifuged at 5,000 rpm for 10 minutes to obtain plasma for assays. Kidneys from each animal were collected separately into formalin containing bottles for histopathology studies.

2.6. Biochemical Assays

Biochemical assays were carried out using plasma collected. The following biochemical assays were performed using the COBAS C111 Chemistry auto-analyzer manufactured by Roches Diagnostics USA: aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), protein (TP), albumin (ALB), urea (URE), creatinine (CR), potassium (K^+), sodium (Na^+), chlorine (Cl^-) and bicarbonate (HCO_3^-).

2.7. Haematological Analysis

A full blood count was conducted on the whole blood collected into EDTA bottles for immediate analysis using the SYSMEX KX-21N automated hematology analyzer (Sysmex Corporation, Kobe, Japan). White blood cell count (WBC), red blood cell count (RBC), haemoglobin concentration (HB), haematocrit (HCT), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and differential leukocyte counts were determined.

2.8. Histopathological Study

The kidneys harvested were rinsed with water and fixed in 10% (v/v) formaline. This was processed routinely by having slices of the organ which was allowed to go through different alcohol concentration in ascending order (70%, 90%, 95%, and 100%) and then, xylene to remove alcohol and prepare the tissues for waxing. It was then embedded in paraffin wax. 5-6 µm sections were sliced from the tissue using a microtome which were mounted on slides and stained with haematoxylin and eosin (HE) [4].

2.9. Statistical Analysis

The statistical analysis was done using Graph pad prism 7.0. The results were reported as mean ± SEM (standard error of mean). The data collected were subjected to Analysis of Variance (ANOVA) to test for the variations of the different parameters observed in the study. Test of significance was at 0.05% probability ($p<0.05$).

3. Results

3.1. Body Weight Changes

The effect of *P. amarus* leaf extract on weekly body weight changes of rats with ibuprofen-induced nephrotoxicity is presented in Table 1. There was no significant ($p>0.05$) body weight gain observed in ibuprofen-induced group of rats and groups treated with different doses of *P. amarus* extract after 28 days treatment compared to the control. Significant ($p<0.05$) weight gain was noticed in rats treated with termisartan drug compared to the control after treatment for the same period.

Table-1. Effect of *P. amarus* on weekly change in weight of rats with ibuprofen-induced nephrotoxicity

	Group I	Group II	Group III	Group IV	Group V	Group VI
Weight wk1 (g)	197.95±5.02^a	211.40 ± 21.88^a	229.64±19.30^a	189.25±8.95^a	191.26±5.36^a	193.74±18.35^a
Weight wk2 (g)	206.25±3.94^a	211.63±19.99^a	226.50±24.89^a	190.08±9.63^a	193.25±4.97^a	195.78±17.41^a
Weight wk3 (g)	202±4.76^a	210.28±20.02^a	216.75±23.34^a	182.98±9.24^a	189.01 ± 5.08^a	191.29±16.06^a
Weight wk4 (g)	210±7.53^a	215.83±16.13^a	223.25±18.17^a	183.13±12.09^a	190.25±6.34^a	192.68±14.14^a

Results were expressed as mean ± SEM of 4 rats. Means of the same column with different letters differ significantly ($P<0.05$). I = 5 ml/kg/day of distilled water; II = 200 mg/kg of ibuprofen (single dose); III = 200 mg/kg of ibuprofen (single dose) + 0.097 mg/kg/day of termisartan drug;

IV = 200 mg/kg of ibuprofen (single dose) + 50 mg/kg/day of AEPA; V = 200 mg/kg of ibuprofen (single dose) + 100 mg/kg/day of AEPA; VI: 200 mg/kg of ibuprofen (single dose) + 200 mg/kg/day of AEPA s

3.2. Changes in Biochemical Parameters and Electrolytes

Results of the effects of *P. amarus* leaf extract on plasma biochemical parameters of rats with ibuprofen-induced nephrotoxicity is shown in Table 2. Induction of rats with 200 mg/kg b.wt. ibuprofen caused a significant ($p<0.05$) increase in the levels of plasma creatinine, AST, ALP, and not significant ($p>0.05$) increase in urea whereas the levels of total protein was significantly ($p<0.05$) decreased while ALT and Albumin were not significantly reduced compared to the control. However, post-treatment of rats with various doses of *P. amarus* leaf extract and termisartan drug significantly ($p<0.05$) reduced the levels of creatinine, AST, ALP and significantly ($p<0.05$) increased ALT, total protein and albumin levels compared to the ibuprofen-induced rats group. These changes noticed were dose dependent. Table 3 shows the effect of *P. amarus* on electrolytes of rats with ibuprofen-induced nephrotoxicity. There was no significant change in electrolytes of rats treated with either termisartan drug or doses of *P. amarus* after 28 days compared to the control.

Table-2. Effect of *P. amarus* on plasma biochemical parameters of rats with ibuprofen-induced nephrotoxicity

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
Urea (mmol/L)	6.74 ± 0.99^a	7.77 ± 0.16^a	9.07 ± 0.44^b	8.91 ± 0.32^a	7.75 ± 0.24^a	7.54 ± 0.26^a
Creatinine (umol/L)	46.37 ± 3.16^a	64.53 ± 2.73^b	37.65 ± 5.27^a	44.40 ± 0.65^a	46.07 ± 2.73^a	48.67 ± 0.27^a
AST (U/L)	112.20 ± 11.18^a	181.66 ± 5.60^b	135.17± 32.28^a	88.46 ± 1.07^a	133.13± 17.55^a	103 ± 0.57^a
ALT (U/L)	39.50 ± 5.85^a	38.33 ± 0.82^a	43.75 ± 11.35^a	40.33 ± 0.88^a	44.25 ± 2.17^a	32.33 ± 6.23^a
ALP (U/L)	102.50 ± 7.89^a	160 ± 5.03^b	130 ± 20.66^b	90.33 ± 0.88^a	109.25±11.35^a	106.33 ± 4.91^a
Total_protein (g/L)	71.23 ± 1.74^a	59.33 ± 2.38^b	60.85 ± 3.28^a	60.86 ± 0.43^a	71.75 ± 4.17^a	69.30 ± 0.82^a
Albumin (g/L)	33.07 ± 4.15^a	28.13 ± 0.83^a	30.35 ± 3.81^a	34.23 ± 1.79^b	37.83 ± 3.68^b	38.26 ± 2.17^b

Results were expressed as mean ± SEM of 4 rats. Means of the same row with different letters differ significantly ($P<0.05$). I = 5 ml/kg/day of distilled water; II = 200 mg/kg of ibuprofen (single dose); III = 200 mg/kg of ibuprofen (single dose) + 0.097 mg/kg/day of termisartan drug; IV = 200 mg/kg of ibuprofen (single dose) + 50 mg/kg/day of AEPA; V = 200 mg/kg of ibuprofen (single dose) + 100 mg/kg/day of AEPA; VI: 200 mg/kg of ibuprofen (single dose) + 200 mg/kg/day of AEPA

Table-3. Effect of *P. amarus* on plasma electrolytes of rats with ibuprofen-induced nephrotoxicity

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
K (Mmol/L)	3.54 ± 0.31	3.28 ± 0.17	4.12 ± 0.16	4.64 ± 0.08	4.56 ± 0.51	3.69 ± 0.19
Na (Mmol/L)	144.77± 5.68	138.47 ± 0.41	134.77± 1.77	154 ± 3.61	138.97 ± 3.49	137.33 ± 1.45
Cl (Mmol/L)	110.67± 5.14	108.27 ± 0.62	103.30 ± 1.07	112.67± 5.46	104.20 ± 1.36	106.10 ± 2.01
HCO_3 (Mmol/L)	16.03 ± 0.86	15.23 ± 0.58	18.05 ± 1.27	13.13 ± 0.38	13.23 ± 0.81	14.33 ± 1.76

Results were expressed as mean ± SEM of 4 rats. Means of the same row with different letters differ significantly ($P<0.05$). I = 5 ml/kg/day of distilled water; II = 200 mg/kg of ibuprofen (single dose); III = 200 mg/kg of ibuprofen (single dose) + 0.097 mg/kg/day of termisartan drug; IV = 200 mg/kg of ibuprofen (single dose) + 50 mg/kg/day of AEPA; V = 200 mg/kg of ibuprofen (single dose) + 100 mg/kg/day of AEPA; VI: 200 mg/kg of ibuprofen (single dose) + 200 mg/kg/day of AEPA. K = potassium; Na = sodium; Cl = chloride; HCO_3 = bicarbonate.

3.3. Changes in Haematological Indices

The effect of *P. amarus* leaf extract on haematological indices of rats with ibuprofen-induced nephrotoxicity is represented in Table 4. Induction of nephrotoxicity in rats with the oral administration of 200 mg/kg b.wt. ibuprofen showed no significantly ($p>0.05$) alterations in many of the haematological indices except the differential leucocyte counts compared to the control. However, the levels of RBC, HB, HCT and PLT in post-treated rats with 50 mg/kg b. wt. *P. amarus* for 28 days were observed to be significantly ($p<0.05$) reduced whereas the levels of the differential counts, monocytes and neutrophils were significantly ($p<0.05$) increase with 200 mg/kg b. wt. *P. amarus* treatment compared to the ibuprofen-induced group of rats. No significant ($p>0.05$) alterations were observed in other indices such as WBC, MCV, MCH, MCHC and lymphocytes. Likewise, there was no significant ($p>0.05$) alterations in the levels of haematological indices in a group of rats treated with 0.097 mg/kg b. wt. termisartan drug after 28 days treatment compared to the control.

Table-4. Effect of *P. amarus* on haematological indices of rats with ibuprofen-induced nephrotoxicity

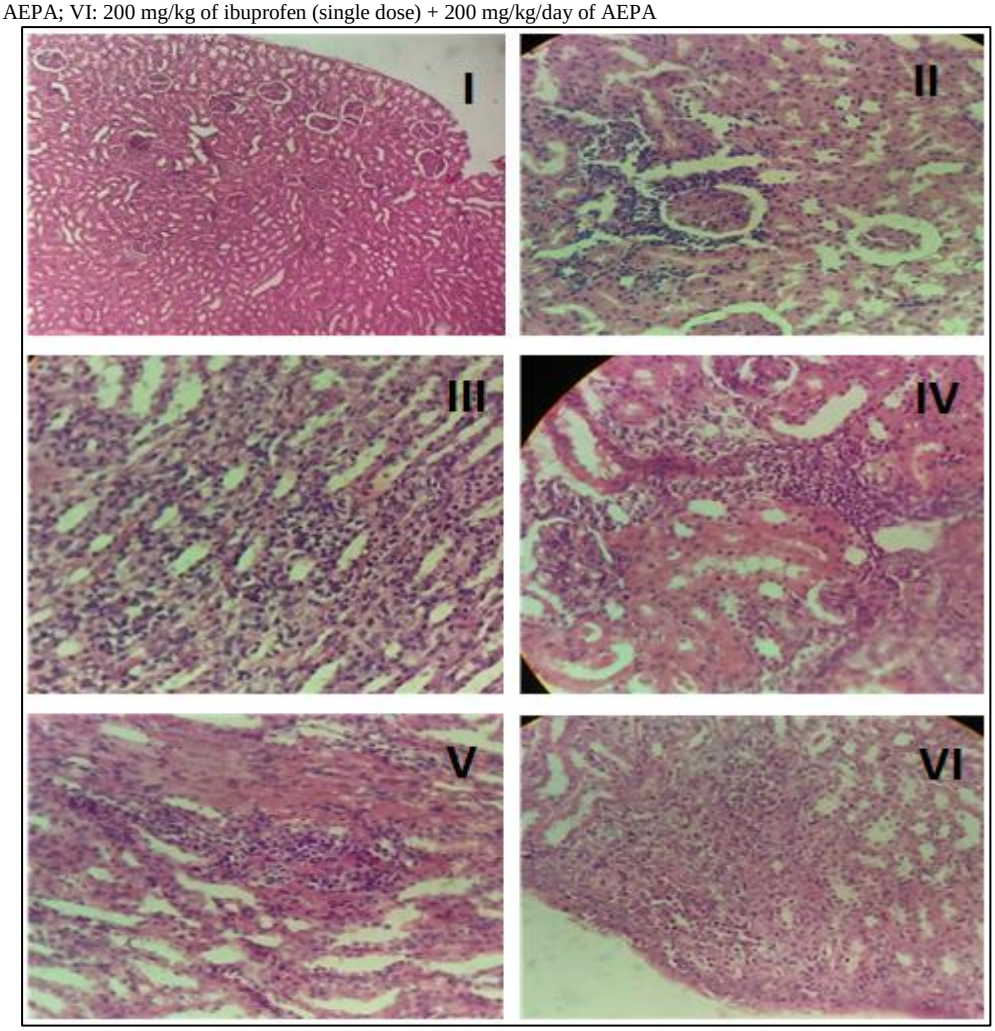
Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
WBC (x10^9/l)	3.87 ± 0.79	5.00 ± 0.05	5.57 ± 1.38	3.50 ± 0.55	3.60 ± 0.47	4.70 ± 0.11
RBC (x10^{12}/l)	7.58 ± 0.61a	7.53 ± 0.14a	7.21 ± 0.62a	3.65 ± 0.24b	8.23 ± 0.24a	8.02 ± 0.26a
HB (g/l)	13.95 ± 0.88a	13.43 ± 0.20a	12.67 ± 0.80a	5.73 ± 0.06b	15.07 ± 0.31a	14.73 ± 0.24a
HCT (%)	47.22 ± 3.41a	46.06 ± 0.44a	42.25 ± 3.33a	19.10 ± 0.51b	51.37 ± 1.30a	52.93 ± 3.02a
MCV (fl)	62.40 ± 0.92	62.36 ± 0.28	58.62 ± 1.52	56.66 ± 0.81	62.50 ± 0.38	62.13 ± 1.24
MCH (pg)	18.50 ± 0.57	18.30 ± 0.231	17.65 ± 0.51	18.46 ± 0.35	18.47 ± 0.23	19.66 ± 0.66
MCHC (g/l)	29.65 ± 0.71	30.50 ± 0.26	30.10 ± 0.56	32.03 ± 0.13	29.47 ± 0.18	30.43 ± 0.17
PLT (x10^9/l)	1046.75 ± 28.72a	939.33 ± 23.31a	946.75 ± 47.71a	488.33 ± 30.02b	1011 ± 77.51a	1012 ± 2.31a
LYM (%)	74.17 ± 5.29	58.23 ± 1.12	55.95 ± 3.88	72.80 ± 3.58	79.15 ± 4.09	59.90 ± 0.91
MON (%)	6.90 ± 4.09a	21.03 ± 0.24b	23.47 ± 6.55b	6.86 ± 2.15a	7.25 ± 2.22a	32.80 ± 1.44b
NEUT (%)	18.92 ± 4.17	20.56 ± 0.26	20.57 ± 5.59	22.03 ± 0.62	14.62 ± 1.78	21.46 ± 0.78

Results were expressed as mean ± SEM of 4 rats. Means of the same row with different letters differ significantly ($P<0.05$). I = 5 ml/kg/day of distilled water; II = 200 mg/kg of ibuprofen (single dose); III = 200 mg/kg of ibuprofen (single dose) + 0.097 mg/kg/day of termisartan drug; IV = 200 mg/kg of ibuprofen (single dose) + 50 mg/kg/day of AEPA; V = 200 mg/kg of ibuprofen (single dose) + 100 mg/kg/day of AEPA; VI: 200 mg/kg of ibuprofen (single dose) + 200 mg/kg/day of AEPA. WBC = white blood cell count; RBC = red blood cell count; HB = haemoglobin concentration; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelet count; LYM = lymphocytes; MON = monocytes; NEUT = neutrophils

3.4. Kidney Histopathology

Histopathological architecture of the kidney showing the effects of *P. amarus* treated rats in ibuprofen-induced nephrotoxicity is represented in figure 1(a-f). Figure 1a shows the control group with no alteration. Administration of ibuprofen in rats induced morphological architectural alterations in the kidney, where proliferation of glomeruli and tubular inflammations were observed (Figure 1b). Treatment with termisartan drug showed minor effect on the kidney (Figure 1c), proliferations of glomeruli and tubular inflammation were also evident. However, the effects of treatment with doses of 50, 100 and 200 mg/kg b. wt. of *P. amarus* on kidney histology are shown in Figure 1d, 1e and 1f respectively. The induced morphological damage by ibuprofen were observed to be remedied with increased doses of *P. amarus* thereby ameliorating the effect of ibuprofen-induced toxicity on the kidney.

Figure-1. Photomicrographs showing the effects of *P. Amarus* on kidney of rats with ibuprofen-induced nephrotoxicity at x400 magnification. I = 5 ml/kg/day of distilled water; II = 200 mg/kg of ibuprofen (single dose); III = 200 mg/kg of ibuprofen (single dose) + 0.097 mg/kg/day of termisartan drug; IV = 200 mg/kg of ibuprofen (single dose) + 50 mg/kg/day of AEPA; V = 200 mg/kg of ibuprofen (single dose) + 100 mg/kg/day of AEPA; VI: 200 mg/kg of ibuprofen (single dose) + 200 mg/kg/day of AEPA



4. Discussion

Phyllanthus amarus has been found to be traditionally useful in several health problems such as diarrhoea, dysentery, jaundice, fevers, scabies, wounds and urinogenital disorders. It is also used in the treatment of kidney problems, urinary bladder disturbances and gonorrhea [45]. Renal disease, among other ailments, has been considered as the 9th major cause of mortality across the world, and is also considered as the sole clinical sign of the disease [2]. This disease is known to reduce excretory function by the kidney, and therefore reduced glomerular filtration rate (GFR), with abnormal homeostasis in blood chemistry [2]. Many pharmaceutical drugs, including ibuprofen have been reported to pose a threat by attacking the kidneys. Investigation of the various biochemical parameters in this study is to assess the ameliorative effect of the *P. amarus* leaf extract in ibuprofen-induced nephrotoxic animals.

It was reported that the methanol extract of *P. amarus* leaves (50-800 mg/kg b.wt.) possessed hepaprotective, nephroprotective and cardioprotective properties by causing a significant dose dependent decrease of total cholesterol, urea, total protein, uric acid, and prostatic, alkaline and acid phosphatases, aspartate transaminase (AST) and alanine transaminase (ALT) levels [41]. At the same time, Adeneye and colleague studied the protective effects of aqueous extracts of *P. amarus* leaves and seeds (100-400 mg/kg/day) in acetaminophen and gentamicin-induced nephrotoxic Wistar rats for 14 days and reported that the plant attenuated elevations in the serum creatinine and blood urea nitrogen levels occasioned by acetaminophen and gentamicin in dose related fashion [53]. The diseased or damaged tissues may release their enzymes into the serum or plasma which become measurable, through altered

cell membrane of the rat organs [54]. In this study, 70% alcohol extract of *P. amarus* leaf (50-200 mg/kg/day) for 28 days was observed to ameliorate the effect caused in ibuprofen-induced nephrotoxic rats. The extract caused a significantly ($p<0.05$) reduction in the levels of creatinine, AST and ALP that were elevated by ibuprofen induction. The extract also significantly ($p<0.05$) reversed the increased levels of ALT, total protein and albumin caused by ibuprofen. These changes were observed to be dose dependent. No significant change was observed in the status of the electrolytes levels in nephrotoxic rats after treatment with extract for 28 days. Kidney plays a major role in drug excretion and detoxification which makes it an important target for toxicological response [55]. Exposing kidney to a high level of drug or/metabolites causes cell damage due to high blood flow, clearance and xenobiotics metabolism [56]. The major kidney damage indicators are creatinine and serum electrolytes. These parameters if altered in the organ will impair the normal functioning of the organ. Creatinine is the major catabolic products of the muscle. The dose dependent reduction in creatinine with the extract suggests the protective ability of the extract on the kidney tissue. The absence of significant change in the plasma levels of electrolytes like potassium, sodium, chloride and bicarbonate, and other biochemical parameter such as urea suggest that the secretory ability and normal functioning of this organ in relations to these parameters were not affected.

Evaluation of haematological indices is also very useful to explain the blood relating functions of plant extracts and/or products [57]. This assessment is relevant, as changes in the haematological system have higher predictive value for human toxicity, when the data are translated from animal studies [58]. Based on the results of the haematological assay, the levels of RBC, HB, HCT and PLT were significantly low at 50 mg/kg of extract while no significant change was observed at doses of 100 and 200 mg/kg extract. It could be that the lower dose of extract may be too low to allow enough response to the influence of ibuprofen on these parameters. However, destruction of RBCs or its decreased production in the bone marrow causes anaemia while HB, RBC and HCT are associated with the total population of red blood cells. The absence of significant change in these parameters at higher doses of extract may suggest that the balance between the rate of production and destruction of the blood corpuscles (erythropoiesis) was unaltered. Increase in WBC indicates boost in immune system while MCHC, MCH and MCV relates to individual red blood cells. No significant alterations observed in WBC, MCV, MCH, MCHC and lymphocytes whereas 200 mg/kg extract significantly increased the levels of monocytes and neutrophils. The absence of significant effect on WBC and lymphocytes, and significant increase in monocytes and neutrophils may suggest a kind of selective immune stimulant property because these are effector cells in the immune system.

The uses of *P. amarus* traditionally for kidney stones and gall bladder stones have been validated by clinical research. *P. amarus* extract was reported to exhibit a potent and effective non- concentration dependent inhibitory effect on calcium oxalate crystal formation which is the building blocks of most kidney stones [51, 59]. The pathology of the kidney in this study showed that the control has no alteration in morphological architecture. However, alterations induced by ibuprofen were observed to be reversed in dose dependent fashion by the different doses of the 70% alcohol extract of *P. amarus* leaf.

5. Conclusion

Phyllanthus belongs to one of the largest genus in the family Phyllanthaceae that is use constantly in traditional medicines to cure diverse human diseases. In this study, 70% alcohol extract of *P. amarus* leaf was use to evaluate the influence of the plant on biochemical and haematological status of rats with ibuprofen-induced nephrotoxicity. The results obtained indicate that the extract of *P. amarus* was able to attenuate majority of the biochemical and haematological parameters in ibuprofen-induced nephrotoxic rats while histological examinations of the kidneys also corroborated these findings. Therefore, the extract has a selective effect on these parameters examined in the rat.

Conflict of Interest

Authors declare no conflict of interest

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