Nauclea latifolia (Rubiaceae) Stem-bark H*ydro*methanolic Extract Abrogates a Histone Deacetylase Inhibitor - induced Multifocal Toxicity in Albino Rats.

Abstract

Objective: *Nauclea latifolia* (Rubiaceae) stem-bark enjoys wide patronage in ethnomedicine due to multiplicity of usage. Acute and subacute hematological and biochemical toxiciy studies are available in literature but none underpins its ameliorative effect with a histone deacetylase inhibitor (HDAC), valproic acid (VPA) which mediates multifocal toxicity in different histological milieu.

Methods: Subacute exposure of experimental albino rats with a high dose of valproic aci (500 mg/kg) was executed orally one hour before posttreatment with *Nauclea latifolia* stem-bark (NLS) extract in three doses (50, 100, 200 mg/kg) and with another group of rats with reference drug, vinpocetin, 25 mg/kg daily for 28 consecutive days after which hematological and biochemical analyses were executed. The liver, kidney and lungs were abstracted for histopathological evaluation.

Results: The HDAC inhibitor, Valproic acids induced multifocal biochemical insults on liver function enzymes, lipid profiles, electrolytes and kidney function which were dose- dependently and significantly ($P < 0.05 – 0.001$) abrogated by the varying doses of NLS extract administered. On the histology the NLS extract effects corroborated the biochemical study in the liver and kidney. The NLS did not demonstrate significant toxicological impingement on the hematology and did not alter VPA-induced histomorphological injury in the lungs cytoarchitecture. The referenced drug, vinpoccetine was unresponsive to VPA-induced alteration in all the tissues investigated in the administered posology.

Conclusion: The NLS extract was effective in abrogating toxicological insults in the liver and kidney but not in the lungs. Further studies are required to understand the mechanism of pharmacological effects of NLS extract and the differential in tissue response.

Keywords: *Nauclea latifolia*, subacute, hydromthanolic extract, valproic acid, vinpocetin, toxicological profile

1. INTRODUCTION

Drug's short- and long-term functional and morphologic adverse effects is evaluated via *in vitro* and *in vivo* animal toxicity studies [1, 2, 3]; Steinberg, 2017). The toxicity studies may consist of acute toxicity studies, subacute or sub-chronic toxicity studies, chronic toxicity studies, special toxicity studies, carcinogenicity studies, genotoxicity studies, reproductive toxicity studies, and toxicokinetic studies [4, 5]. Effects that result from a single dose or single exposure of a chemical are referred to as acute toxicity. Acute toxicity studies are accompanied by administering the chemical orally, by inhalation or dermally to determine the dose that causes 50% mortality. The values calculated through the oral and dermal routes of exposure are referred to as Lethal Dose 50 (LD_{50}). The LD_{50} is defined as that amount of chemical essential to kill 50% of the test animals in a group within the first 14 days following exposure and Lethal Concentration 50 (LC_{50}) is comparable to the concentration of chemical administered to kill 50% of the animals by inhalation exposure. Results of acute toxicity studies provides, potential target organs of toxicity, an estimation of safe accurate doses for humans, suitable dose for multiple dose studies, depict the potential for acute toxicity in humans, the time course of drug-induced clinical observations and species specific differences in toxicity [4].

Subacute or sub-chronic toxicity studies evaluate a drug's toxicity potential and pathological effects through a period of fourteen to ninety days [4]. Subacute and sub-chronic differ in duration of exposure. Subacute systemic toxicity is defined as adverse effects occurring after multiple or continuous exposure between 24 h and 28 days. Sub-chronic systemic toxicity is defined as adverse effects occurring after the repeated or continuous administration of a test sample for up to ninety days or not exceeding 10% of the animal's lifespan [4]. Either in acute or subacute or sub-chronic or in chronic toxicity studies, data obtained from observed effects includes physical examinations, body weight, food/water consumption,

mortality, hematology, bone marrow, coagulation, organ weights, blood chemistry, urinalysis, gross pathology, and histopathology are collected and statistically analyzed [4].

Drugs are the molecules used as medicines or as components in medicines to diagnose, cure, mitigate, treat, or prevent diseases [6]. Many drugs have toxicity implications most especially when administered at high doses. One of such drugs with widely reported toxicity is HDAC inhibitor, sodium valproate or valproic acid. VPA-induced global toxicity relating to neurotoxicity [7], hepatotoxicity [8], hematotoxicity [9], nephrotoxicity [10], pancreatitis [11], bone marrow suppression [12], teratogenicity and developmental toxicity [13, 14] and numerous idiopathic effects which in the offspring might lead to autistic spectrum disorder [15, 16]. Sodium valproate is 2-propylpentanoic acid, first marketed about 45 years ago for the management of epilepsy [17], is generally prescribed mood stabilizer and anti-convulsant used to control generalized and partial seizures [18] . Afterward, valproate was discovered to relieve HDAC-dependent transcriptional repression and to induce histone hyperacetylation both *in vitro* and *in vivo* [19, 20] by acting as a pan-inhibitor of HDAC classes I and IIa: inhibiting HDAC 1, 2, 3, 4, 5, 7 and 8 with varying potencies [21].

Though the conjoint adverse effect related with VPA are classically benign, less common adverse effect which may occur includes hepatotoxicity, teratogenicity and acute pancreatitis (AP). The mechanisms of toxicity reported are VPA-induced hyperammonemic encephalopathy (VHE) [22] and induction of oxidative stress in the kidney [23], brain [24] and hepatocytes [25].

Medicinal plants with antioxidants effects have been reported [26, 27] and among these plants, great attention has been paid to members of the large family of the Rubiaceae, including the genus Nauclea, which is composed of about 35 species spread worldwide. The taxon *Nauclea latifolia* Sm., commonly known as the African peach or African pincushion tree, is widely distributed through Sub-Saharan regions. This plant grows as an evergreen shrub or small tree present in wooded savannah and in the margins of tropical forests. The stem, bark, root, leaf and fruit are traditionally used by local populations

in ethnomedicine and long-standing use of *N. latifolia* has been reported in Central and West Africa. The ethnomedical applications cover a large range of diseases, including pain-management, diabetes, infections, and malaria [28, 29]. *Nauclea latifolia* has been found to exert anti-hepatotoxic, hypoglycemic, anti-nociceptive, anti-inflammatory anti-pyretic and other numerous activities in animal models [30].

Alkaloid rich extracts from the *Nauclea latifolia* is reported to interact *in vitro* with DNA of bacteria and mammalian cells, leading to G2-M cell cycle arrest and heritable DNA-damage and provoke single-strand breaks in liver, kidney and blood cells [31]. But, more recently, it has been reported that repeated administration for 28 days of high doses (200, 400, 600 and 800 mg/kg body weight) of NLS aqueous stem may lead to damage in the kidney and liver [32].

Though the NLS acute and subacute toxicity profile on hematological and biochemical profiles have been reported [30, 32] but the effect of NLS the histopathology (of liver, kidney and the lungs) and on the multifocal toxicity - induced by VPA is yet to be reported. This study seek to update the existing gap and provide information potential ameliorative effect of subacute administration of NLS extract on the multifocal toxicity induced by VPA in rats by evaluating the hematology, serum chemistry and reveal the histopathology of the liver, kidney and lungs.

2. MATERIALS AND METHOD

2.1 **Drugs, Chemicals and equipment**

The study protocol utilizes: Sodium valproate (Epilim, Sanofi, France), Vinpocetine (Cognitol, Tyonex, Nigeria) both purchased from Sicone Pharmacy (Nigeria). Limited, Rivers State, Nigeria. Methanol 99.8% (Lobal Chemie, Mumbai, India), *n*- hexane (extrapure 85%) (Lobal Chemie, Mumbai, India), Diethyl ether (Lobal Chemie, Mumbai, India), formalin (Lobal Chemie, Mumbai, India). The equipment utilized includes: rotary evaporator (Shenke® R-205, Shangai Shenshun Biotechnology Co. Ltd, China), analytical balance (AR323 CN) Ohaus Corp. Pine Brook, NJ, USA), auto-hematology analyzer model MY-B002B (Maya Medical Equipment Limited, China), Spectrophotometer model SM-23 D (Surgifield Medical, England), scientific weighing balance model TH 600 (Labscience, England), centrifuge model 412B (Techmel and Techmel, USA), Water bath (Techmel and Techmel, USA).

2.2 Collection and Authentication of plant materials

The stem bark of *Nauclea latifolia* were collected in Uyo, Akwa Ibom state and supplied dried by Mr. Okon Etefia, a traditional herbalist, attached to Pharmacognosy Department, University of Uyo, Nigeria and the plant was authenticated by Dr Oladele Adekunle, a taxonomist attached to the Forestry Department at university of Port Harcourt, Nigeria. The Herbarium specimen with voucher number UUPH 20(a) is deposited at Department of Pharmacognosy, University of Uyo, Akwa Ibom State, Nigeria.

2.3 Preparation of *N. latifolia* stem-bark (NLS) extract

The dried *N. latifolia* stem bark (NLS) was pulverized to fine particles using mechanical grinder. A 250 g weight of stem bark powder was then macerated in 2000 mL of *n*-hexane for defatting. After 24 hours of maceration, the extract was concentrated using a rotary evaporator and the marc submerged in 2000 mL of methanol. It was macerated for 72 hours while shaking vigorously every 2 hour for 12 hours. Rotary evaporator was used to concentrate the extract obtained after which it was evaporated to dryness on the water bath at 45°C. The percentage yield was then calculated. Phytochemical screening of the plant extract was carried at the Pharmacognosy and Phytotherapy Department laboratory, University of Port Harcourt. The bioactive agents screened include: triterpenoids, cardiac glycosides, flavonoids, alkaloids, saponins, and phlobatannins using standardized protocol [33].

2.4 Animals

Forty male Wistar albino rats weighing about 150 – 170 g were obtained from the animal house, Department of Pharmacology and Toxicology, University of Nigeria Nsukka. The animals were acclimatized in the University of Port Harcourt Animal House for 14 days under standard laboratory conditions before commencement of the experiment. The average relative humidity and ambient temperature were 40 – 55% and 26ºC respectively. The animals were sustained under normal light and dark cycles. The rats were housed in plastic cages and fed with pelleted rodent chow (Vital Feeds, Edo state, Nigeria) and allowed unfettered access to water *ad libitum*. The experimental protocol was in line with institutional guideline for care and use of animals for experiment as specified in Guide to the Care and Use of Animals in Research and Teaching (NIH, 1996) with University of Port Harcourt Animal ethics committee approval (No. UPHAEC/2018/008).

2.5 Acute toxicity study

The LD_{50} of NLS has already be estimated to be 850 mg/kg [34] in Wistar albino rats and 1414.2 mg/kg body weight in Swiss albino mice [35]. The dose levels 50, 100 and 200 mg/kg in this study was derived from 1/5, 1/10 and $1/20^{th}$ of LD_{50} dose in rats.

2.6 Experimental procedure

Valproate induced toxicity: Doses and treatment

Sodium valproate or valproic acid (VPA) brand Epilim® formulated as 300 mL syrup was used to induce multifocal toxicity at a dose 500 mg/kg daily for 30 days when administered orally by gavage in the experimental animals [36]. Each one mL contains 200 mg sodium valproate.

The animals were divided into six groups with 7 animals per group by randomization adopting block permuted plan. The NLS, valproate and vinpocetin (Cognitol®) (the reference drug) were administered orally per kg of body weight once daily for 30 days. Sodium valproate (500 mg/kg) was administered one hour prior to the administration of the control drugs or extracts respectively for animals in groups 2 to 6. The NLS and vinpocetin 25 mg/kg were solubilized in 2% Tween 80 (Polysorbate 80). The various experimental groups utilized for the study are as follows:

Group 1 (negative control): The animals in this group received 2% Tween 80 in 10 ml/kg distil water.

Group 2 (disease control group) : The animals in this group received sodium valproate followed by 2% Tween 80 in 10 ml/kg distil water

Group 3 to 5 (test groups): The animals in these groups received sodium valproate (500 mg/kg) followed by the NLS extract 50 mg/kg, 100 mg/kg and 200 mg/kg respectively.

Group 6 (reference control): The animals in this group received sodium valproate followed by vinpocetine 25 mg/kg.

The rats were administered valproic acid (500 mg/kg b.w) and one hour later, either distil water, NLS or vinpocetin was administered adopting standard procedure [36] .

Hepatotoxicity and nephrotoxicity percentage (%) was deduced using this formular $=$
$\left[\left(\frac{\text{VA—W(negative control)}}{\text{W(negative control)}}\right) \times 100\right]$

Hepatoprotective and nephroprotective activity (%) was calculated as follows:

$$\text{Protective activity (\%)} = \left[1 - \left(\frac{\text{NLS} - \text{W}}{\text{VA} - \text{W}}\right)\right] \times 100$$

Where, NLS, VA, and W are experimental variables estimated in the rats treated with valproic acid plus NLS (Test groups), valproic acid (diseases control group) and distil water treated animals (negative control) respectively.

2. 8 **Evaluation of weights**

The initial weights of the rats were recorded followed subsequently with daily weights monitored for 30 days and the final weights before sacrificing the animals were recorded. The final body weights and the weights of internal organs such the brain, the heart, the liver, the lungs, the kidney, the stomach, the spleen, the ovary and the testes were exsanguinated and weighed and the relative organ weights calculated.

2.9 Blood sampling

At the close of overnight fasting, the animal was anaesthetized using diethyl ether and blood was obtained by dissecting the jugular vein with a sharp surgical blade. Samples of blood were collected into EDTA (ethylene diamine tetraacetic) bottles for hematology and lithium heparin container for biochemical assays.

The blood in the lithium heparin container was allowed to clot at room temperature for serum formation and centrifuged for 5 minutes at 3000 r / minutes before serum was then collected using micro pipette into vercutainers, which were preserved at –20 ºC until used for various biochemical analysis such as: liver function test, antioxidant, lipid profile and kidney function.

2.10 Hematological analysis

This was carried out using an automated hematology analyzer model MY-B002B (Maya Medical Equipment Limited, Beijing, China). The various hematological parameters analyzed include: PCV (packed cell volume), HGB (hemoglobin), WBC (total white blood cell), PLT (platelet), RBC (total red blood cell), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), NEU (neutrophils), LYM (Lymphocytes), MEB (monocytes), MID (mid-range percent of monocytes, eosinophils, and basophils), MPV (mean platelet volume), RDW-SD (red cell distribution width- standard size), RDW- CV (red blood cell distribution width – coefficient of variation), RDW (red cell distribution width (P-LCR). The analysis followed earlier reported procedure [35].

2.1 Biochemical serum analysis

Biomarkers of toxicity evaluations were evaluated using established protocols. The levels of serum liver enzymes, serum alanine transaminase (ALT) and serum aspartate transaminase (AST) were evaluated by the method of Reitman and Frankel [37]; alkaline phosphatase (ALP) was reported by the procedure of Roy [38]. Colorimetric assays were used to determined serum total bilirubin (TBIL) and conjugated bilirubin (CBIL) by the methodology of Jendrassik and Grof [39], total protein by Biuret procedure of Flack and Woollen[40], albumin by Doumas et al. [41], high density lipoproteins (HDL) profile by the protocol of Lopes-Virella [42]; total cholesterol (TCHO) was obtained by the procedure of Allain and Roeschlau method [43]; triglycerides (TG) by Burtis and Tietz, [44]; and electrolytes such as sodium (Na^+) by the procedure of Maruna [45] and, Suderman and Delory [46], chloride (Cl^-) by the colorimetric procedure of Schoenfeld and Lewellen [47], potassium (K^+) and bicarbonate (HCO_3^{2-}) by the methodology of Henry et al., [48]. Serum creatinine and serum urea were determined by the method of Varley and Alan [49].

2.12 Histopathology studies

Prefrontal cortex brain tissue was fixed in 20 % formal saline while other tissues harvested such as liver, lungs and kidneys were fixed in 10 % formal saline.

The tissues, lungs, liver, and kidney harvested from the control and test experimental albino rats were fixed in 10 % formal saline for 48 h. Tissue specimens were then dissected and representative tissue was collected for standard processing into paraffin-embedded tissue blocks. Tissue sections, 4 mm, were cut using a rotary microtome model Leica RM2125 RT. Staining was optimized with haematoxylin and eosin protocol [50] on all slides. Morphological changes were examined in well stained slides under a light microscope after mounting in a mixture of distyrene (a polystyrene), plasticizer (tricresyl phosphate) and xylene, generally called DPX mountant (Atom Scientific, Manchester, UK).

2.13 Statistical analysis

The data analysis was done by Graph pad Prism 5.1 using one-way analysis of variance (ANOVA) and expressed as Mean ± SD. Multiple comparison among groups were made according to the Turkey's test. *P* values < 0.05 were considered significant.

3. RESULTS

3.1 Phytochemical screening

The phytochemical analysis of the extract is summarized in the Table 1 below. The *Nauclea laifolia* stem-bark extract (NLS) demonstrated the presence of saponins, flavonoids, glycosides, tannins, phlobatannins and cardiac glycosides. Alkaloids, triterpenoids and anthraquinones were absent.

Table 1: Phytochemical screening of *Nauclea laifolia* stem-bark extract.

Constituents	Test	Observation
Alkaloids	Mayer Hager Wagner Dragendorff	- - - -
Saponins	Frothing Emulsion	- +
Flavonoids	Schinoda	+
Glycosides	Fehling's Molisch	+ +
Tannins	Ferric chloride	+
Phlobatannins	1% HCl	+
Triterpenoids	Liebermann Burchard	-
Cardiac glycosides	Keller-Kiliani Salkowski	+ +
Anthraquinones	Free Combined	- -

Key: (-) Represents absence; (+): represents presence.

3.2 Effect on body weights

The effects on body weights are evaluated in Table 2 below. No treatment or intoxication related adverse-effect on body weight was observed from his study. There was significant increase (P <0.001) in the body weights of the animals when compared with the initial weighs at the start of the experiment. Comparison of the disease control group with the normal control showed a significant reduction in body weight of the rats during the course of the experiment.

Table 2: Effects NLS extract on valproic acid induced intoxication on body weights of experimental rats following continuous oral sub-acute dosing for 4 weeks

	Weights (g)					
Group	**Initial Weight**	**WK1**	**WK2**	**WK3**	**WK4**	**Final Weight**
Grp 1	134.33±4.99	237±0.07^{c,f}	162.77± 1.65^{c,f}	176.91±1.93^{c,f}	184.67±2.01^{c,f}	186.83±7.22^{c,f}
Grp 2	119±2.24^{cd}	92.82±3.83$^{c,d;c,f}$	136.19±0.57$^{c,d;\ c,f}$	146.09±0.93$^{c,d;c,f}$	147.44±1.15$^{c,d;c,f}$	148.5±4.45$^{c,d;c,f}$
Grp 3	144.43±2.44^{ce}	123.77±0.92$^{c,e;c,f}$	153.27±0.83$^{c,e;\ c,f}$	155.43±0.83$^{c,e;c,f}$	156.59±0.86$^{c,e;c,f}$	153.5±3.02^{c,f}
Grp 4	124.75±2.46	154.75±0.74$^{c,e;c,f}$	145.43±0.35$^{c,e;c,f}$	154.79±0.46$^{c,e;c,f}$	162.5±0.53$^{c,e;c,f}$	161.36±2.59$^{b,e;c,f}$
Grp 5	112±1.37	149±0.07$^{c,e;c,f}$	129±0.53$^{c,e;\ c,f}$	139.48±0.76 $^{c,e;c,f}$	143.11±0.50^{c,f}	141.33±1.07^{c,f}
Grp 6	133± 0.26 ^{c,e}	134.1 ±0.21 $^{c,e;\ c,f}$	146.2±0.33 $^{c,e;\ c,f}$	157.9±0.53 $^{c,e;\ c,f}$	171.6±0.63 $^{c,e\ ;\ c,f}$	180.1±1.37 $^{c,e;c,f}$

Group 1: Negative control receiving 10 mL/kg b.w. 2% Tween 80; Group 2: Diseases control group receiving 10 mL/kg b.w. 2% Tween 80 + valproic acid 500 mg/kg; Group 3 receiving NLS extract (50 mg / kg b.w.) + valproic acid 500 mg/kg; Group 4 receiving NLS extract (100 mg / kg b.w.) + valproic acid 500 mg/kg; Group 5 receiving NLS extract (200 mg / kg b.w.) + valproic acid 500 mg/kg, Group 6: Reference control receiving Cognitol (25 mg/kg b.w.) + valproic acid 500 mg/kg. NLS = *Nauclea latifolia* stem-bark. Values presented as mean ± standard deviation (n = 3 - 7; $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ ^{d}Values are compared with the negative control group, ^{e}Values are compared with the diseases control group, ^{f}values are compared with initial weights using one way ANOVA and Turkey Test.

3.3 Effect on relative organ weights: Relative organ weight was calculated as (organ weight (g)/ body weight of animal on sacrifice day (g) × 100 and the result is presented in Table 3. The study shows the absence of target organ toxicity as there was no significant differences observed across the different treatment groups. There was however a marked reduction in liver weight of the animals in group 3 and an increased stomach weight in group 5 ($P < 0.05$) as compared to the disease control group.

Table 3: The effect of NLS extracts on valproic acid intoxication on relative organ weights of experimental rats following continuous oral sub-acute dosing for 4 weeks.

Organs	Grp 1	Grp2	Grp 3	Grp 4	Grp 5	Grp 6
LIVER	4.40±0.21	4.98±0.82	3.87±0.48[a,d]	4.30±0.53	3.86±0.31	3.83±0.66
KIDNEY	0.65±0.10	0.73±0.09	0.71±0.05	0.77±0.16	0.77±0.22	0.72±0.05
LUNGS	1.38±0.53	1.87±0.15	2.41±0.87	1.68±0.56	1.37±0.15	1.07±0.52
TESTES	2.12±0.24	2.47±0.08	2.40±0.29	2.54±0.09	2.48± 1.43	2.43±0.30
HEARTS	0.44±0.1	0.46 ±0.13	0.40±0.06	0.42±0.06	0.43±0.09	0.45±0.07
BRAIN	0.86±0.09	1.09±0.18	1.04±0.26	0.93±0.10	1.04±0.14	0.86±0.07
STOMACH	2.05±0.14	1.36±0.15	1.63±0.35	1.72±0.43	2.34±0.64[a,d]	1.73±0.53
SPLEEN	0.62±0.31	0.61±0.09	0.47±0.16	0.45±0.23	0.50±0.10	0.42±0.11

Group 1: Negative control receiving 10 mL/kg b.w. 2% Tween 80; Group 2: Diseases control group receiving 10 mL/kg b.w. 2% Tween 80 + valproic acid 500 mg/kg; Group 3 receiving NLS extract (50 mg / kg b.w.) + valproic acid 500 mg/kg; Group 4 receiving NLS extract (100 mg / kg b.w.) + valproic acid 500 mg/kg; Group 5 receiving NLS extract (200 mg / kg b.w.) + valproic acid 500 mg/kg, Group 6: Reference control receiving vinpocetin (25 mg/kg b.w.) + valproic acid 500 mg/kg. NLS = *Nauclea latifolia* stem-bark. Values presented as mean ± standard deviation (n = 3 - 7; [a]$P < 0.05$, [d]Values are compared with the the diseases control using one way ANOVA and Turkey Test.

3.4 Effect on hematology

The effect on hematological indices is shown in Table 4 below. The different treatments and intoxication showed no statistically significant effect on hematological parameters

Table 4: Effects of NLS extract on valproic acid intoxication on hematological parameters of experimental rats following continuous oral sub-acute dosing for 4 weeks.

Parameter	Unit	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6
WBC	10^9/L	19.3±6.2	11.8±5.9	11.3±5.6	14.9±11.7	14.2±3.2	14.5±3.0
LYM	(%)	57.3±12.6	54.3±15.8	52.2±10.2	48.7±10.2	64.1±3.6	63.0±2.2
MID	(%)	10.2±1.1	8.7±3.3	7.7±1.4	9.93±2.8	10.0±1.4	9.63±1.25
NEUT	(%)	32.5±11.6	36.7±13.3	40.1±10.2	41.4±12.2	26.0±2.3	24.6±2.0
RBC	10^{12}/L	5.3±0.1	4.8±0.4	5.3±0.3	5.2±0.8	4.7±1.0	5.23±0.3
HGB	g/dL	13.8±0.7	12.1±1.4	13.5±0.9	13.1±2.0	12.3±2.6	12.8±0.8
PCV	(%)	28.4±0.7	25.9±2.1	28.0±2.0	28.2±4.5	25.2±5.3	35.2±5.8
MCV	fL	53.7±1.5	53.6±0.8	52.8±1.1	54.7±0.6	53.3±0.9	67.3±9.2
MCH	pg	26.1±0.9	24.9±1.0	25.3±1.2	25.2±0.3	25.9±0.4	24.6±1.5
MCHC	g/dL	48.7±2.2	46.7±2.4	48.1±2.5	46.3±0.9	48.7±1.6	35.3±9.8
RDW-SD	fL	37.8±5.7	39.0±0.0	38.8±2.0	40.9±1.5	39.6±3.9	39.5±1.5
RDW-CV	(%)	17.5±2.2	18.2±0.3	18.4±1.0	18.7±0.8	18.5±1.7	18.5±0.5
PLT	10^9/L	404.3±67	402.7±89	379.4±187	301.3±118	399±312	300±62.8
MPV	fL	8.7±0.2	8.3±0.2	8.4±0.5	8.4±0.3	8.5±0.5	8.30±0.4
PDW	(%)	9.4±0.6	9.9±1.2	9.1±1.4	9.4±1.1	8.5±2.1	8.7±0.2
PCT	(%)	0.4±0.1	0.3±0.1	0.4±0.2	0.3±0.1	0.3±0.3	0.32±0.0
P-LCR	(%)	21.3±2.2	16.1±2.1	15.8±5.5	16.5±4.4	13.9±12.5	13.1±0.3

Group 1: Negative control receiving 10 mL/kg b.w. 2% Tween 80; Group 2: Diseases control group receiving 10 mL/kg b.w. 2% Tween 80 + valproic acid 500 mg/kg; Group 3 receiving NLS extract (50 mg/kg b.w.) + valproic acid 500 mg/kg; Group 4 receiving NLS extract (100 mg/kg b.w.) + valproic acid 500 mg/kg; Group 5 receiving NLS extract (200 mg/kg b.w.) + valproic acid 500 mg/kg, Group 6: Reference control receiving vinpocetine (25 mg/kg b.w.) + valproic acid 500 mg/kg. PCV: packed cell volume; HGB: hemoglobin; WBC: total white blood cell; PLT: platelet; RBC: total red blood cell: MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; NEU: neutrophils; LYM: Lymphocytes; MEB: monocytes, eosinophils and basophils.MID: mid-range percent of monocytes, eosinophils, and basophils; , MPV: mean platelet volume, RDW-SD: red cell distribution width- standard size, RDW- CV: red blood cell distribution width – coefficient of variation; RDW: red cell distribution width, P-LCR; NLS = *Nauclea latifolia* stem-bark. Values presented as mean ± SD (n = 3 – 7).One -way Analysis of variance (ANOVA) followed by post hoc Turkey's multiple comparison Test. P > 0.05.

3.5 Effect on liver enzymes

The effect of NLS following subacute intoxication with valproic acid on liver enzymes and other biochemical parameters are summarized in Table 5. The result indicates statistical significant increase in GGT ($P < 0.001$), AST ($P < 0.001$), ALT ($P < 0.001$), ALP ($P < 0.001$), TBIL ($P < 0.05$) and CBIL ($P <$

0.05) in the diseases control group compared to the negative control group; nevertheless, TP ($P < 0.001$) and ALB ($P < 0.05$) decreased. Intoxication of experimental rats with valproic acid induced hepato-cellular damage reveal by significant elevation ($P < 0.05$ - 0.001) in the levels of serum GGT (71%), AST (57%), ALT (113%), and ALP (46%), TBIL (64%) and CBIL (47%) and significant decrease in TP (38%) and ALB (37%) compared to the negative control group. Nevertheless sub-acute pretreatment for 30 days with NLS extract (50 mg/kg) protected the rats against valproic acid induced hepatotoxicity as evidence in the reduction of hepatic biomarkers in the serum. Pretreatment with NLS 50 mg/kg body weight reduces GGT (89%), AST (71%), ALT (105%), ALP (40%), TBIL (108%) and CBIL (153%) and elevated TP (110%) and ALB (103%). Similarly, pretreatment of NLS 100 mg/kg reduces GGT, AST, ALT, ALP, TBIL, CBIL by 104%, 69%, 101%, 49%, 81%, 136% and elevated TP, ALB by 114%, 105%; while pretreatment of NLS 200 mg/kg reduces these indicators by 106%, 68%, 89%, 92%, 88%, 145% and elevated TP, ALB by 122%, 114% respectively compared to the disease control group. The hepatoprotective activity was dose dependent for only GGT, ALT, AST, TP, ALB and CBIL. The least dose of the extract however showed better hepatoprotective effect on AST and ALT than other treatments. Vinpocetine administration also showed slight hepatoprotection. This was evident in the reduction in GGT, AST, ALT, ALP, TBIL and CBIL by 64%, 68%, 107%, 92%, 107%, 112% and elevation in TP 117% and ALB 112%.

Table 5: Effects of valproic acid intoxication and pretreatment with NLS extract on liver enzymes of experimental rats following continuous oral sub-acute dosing for 4 weeks

Parameters	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6
GGT	1.68 ± 0.06	2.87 ± 0.15[c,d] *(71%)*	1.81 ± 0.16[c,e] *(89%)*	1.63 ± 0.20[c,e] *(104%)*	1.61 ± 0.36[c,e] *(106%)*	2.11 ± 0.31[b,e] (64%)
AST	46.33 ± 3.79	72.67 ± 2.52[c,d] (57%)	53.86 ± 3.34[c,e] (71%)	54.50 ± 1.73[c,e] (69%)	54.67 ± 4.16[c,e] (68%)	54.75 ± 2.50[c,e] (68%)
ALT	16.00 ± 4.00	34.00 ± 3.46 [c,d] (113%)	15.00 ± 2.71[c,e] (105%)	15.75± 3.30[c,e] (101%)	18.00 ± 1.00[c,e] (89%)	14.75 ± 3.40[c,e] (107%)
ALP	43.00 ± 3.61	62.67 ± 2.08[c,d] (46%)	54.71 ± 3.45 [a,e] (40%)	53.00 ± 2.45[b,e] (49%)	44.67 ± 2.52[c,e] (92%)	44.50 ± 3.42[c,e] (92%)
TP	67.67 ± 3.01	41.67 ± 6.51[c,d] (38%)	70.29 ± 4.39[c,e] (110%)	71.25 ± 4.50[c,e] (114%)	73.33 ± 1.53[c,e] (122%)	72.00 ± 2.16[c,e] *(117%)*
ALB	38.33 ± 3.512	24.00 ± 3.61[a,d] (37%)	38.86 ±3.24[c,e] (103%)	39.00 ± 2.16[c,e] (105%)	40.33 ± 1.53[c,e] (114%)	40.00 ± 1.63[c,e] (112%)
TBIL	14.00 ± 2.00	23.00 ± 4.10[a,d] (64%)	13.29 ± 1.80[b,e] (108%)	15.75 ± 2.75[a,e] (81%)	15.10 ± 4.15[a,e] (88%)	13.35 ± 2.23[b,e] (107%)
CBIL	8.30 ± 1.28	12.20 ± 1.68[a,d] (47%)	6.24 ± 1.12[c,e] (153%)	6.90 ± 1.16[c,e] (136%)	6.53 ± 1.68[c,e] (145%)	7.85 ± 1.38[b,e] (112%)

Group 1: Negative control receiving 10 mL/kg b.w. 2% Tween 80; Group 2: Diseases control group receiving 10 mL/kg b.w. 2% Tween 80 + valproic acid 500 mg/kg; Group 3 receiving NLS extract (50 mg / kg b.w.) + valproic acid 500 mg/kg; Group 4 receiving NLS extract (100 mg / kg b.w.) + valproic acid 500 mg/kg; Group 5 receiving NLS extract (200 mg / kg b.w.) + valproic acid 500 mg/kg; Group 6: Reference control receiving vinpocetine (25 mg/kg b.w.) + valproic acid 500 mg/kg. NLS = *Nauclea latifolia* stem-bark. GGT: Gamma glutamyl transferase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: Alkaline phosphatase; TP: total protein; ALB: albumin; TBIL: total bilirubin; CBIL: conjugated bilirubin. Values presented as mean ± standard deviation (n = 3 – 7); [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ [d]Values are compared with the negative control group, [e]Values are compared with the diseases control group; all values were compared using one way ANOVA and Turkey Test.

3.6 Effect on lipid profile

The effect of NLS on lipid profile following sub-acute valproic acid intoxication is presented in Table 6. There was statistical significant elevation of TC (P <0.01), TG (P <0.01), LDL (P <0.001) and VLDL (P <0.01) and depression in HDL (P <0.01) in the rats intoxicated with valproic acid compared to the control group as indicated by the result. Intoxication of experimental rats led to imbalance in the lipid profile shown by significant elevation (P <0.01 - 0.001) in the levels of TC (35%), TG (77%), LDL (108%), VLDL (71%) and depression in HDL (33%). However sub-acute treatment of the rats with NLS extract protected against hyperlipidemia induced by valproic acid as demonstrated by the depression of the less dense lipoproteins. Treatment with 50 mg/kg body weight NLS extract for 30 days reduced TC (92%), TG (111%), LDL (48%), VLDL (154%) and elevated HDL (84%). Treatment with NLS 100 mg/kg demonstrated similar effect to reduce TC (112%), TG (109%), LDL (107%), VLDL (132%) and elevate HDL (87%); while treatment with NLS 200 mg/kg reduced these markers by 96%, 85%, 111%, 109% and increased HDL by 109% when compared to the disease control group. The hepatoprotective activity was dose dependent for only TC, HDL and LDL. Vinpocetine treatment also demonstrated significant hepatoprotective activity on lipid profile in depressing TC, TG, LDL, VLDL by 93%, 68%, 65%, 126% and elevating HDL by 63%.

Table 6: Effect of valproic acid intoxication and pretreatment with NLS extract on lipid profile of experimental rats following continuous oral sub-acute dosing for 4 weeks

	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6
TC	2.17 ± 0.15	2.92 ± 0.45[b,d] (35%)	2.23 ± 0.19[b,e] (92%)	2.08 ± 0.05[b,e] (112%)	2.20 ± 0.20[a,e] (96%)	2.22 ± 0.09[b,e] (93%)
TG	0.69 ± 0.11	1.22 ± 0.17[b,d] (77%)	0.63 ± 0.16[c,e] (111%)	0.64 ± 0.02[b,e] (109%)	0.77 ± 0.19[a,e] (85%)	0.86 ± 0.08 (68%)
HDL	1.16 ± 0.07	0.78 ± 0.09[b,d] (33%)	1.10 ± 0.08[b,e] (84%)	1.22 ± 0.11[c,e] (87%)	1.11 ± 0.11[b,e] (116%)	1.02 ± 0.09[a,e] (63%)
LDL	0.67 ± 0.13	1.38 ± 0.09[c,d] (106%)	1.04 ± 0.03[b,e] (48%)	0.62 ± 0.08[c,e] (107%)	0.59 ± 0.13[c,e] (111%)	0.92 ± 0.15[c,e] (65%)
VLDL	0.48 ± 0.03	0.82 ± 0.12[b,d] (71%)	0.29 ±0.07[c,e] (156%)	0.37 ± 0.13[c,e] (132%)	0.45 ± 0.04[c,e] (109%)	0.39 ± 0.09[c,e] (126%)

Group 1: Negative control receiving 10 mL/kg b.w. 2% Tween 80; Group 2: Diseases control group receiving 10 mL/kg b.w. 2% Tween 80 + valproic acid 500 mg/kg; Group 3 receiving NLS extract (50 mg / kg b.w.) + valproic acid 500 mg/kg; Group 4 receiving NLS extract (100 mg / kg b.w.) + valproic acid 500 mg/kg; Group 5 receiving NLS extract (200 mg / kg b.w.) + valproic acid 500 mg/kg, Group 6: Reference control receiving vinpocetine (25 mg/kg b.w.) + valproic acid 500 mg/kg. NLS = *Nauclea latifolia* stem-bark; TC: total cholesterol; TG: triglycerides; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein. Values presented as mean ± standard deviation ($n = 3 – 7$); [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ [d]Values are compared with the negative control group, [e]Values are compared with the diseases control group; all values were compared using one way ANOVA and Turkey Test.

3.7 Effect on kidney profile and electrolytes

The effect of NLS following subacute intoxication with valproic acid on kidney profile markers CR, UA; and electrolytes Na^+, K^+, Cl^- and HCO_3^- were summarized in Table 7. There was statistical significant increase in UA ($P < 0.001$), CR ($P < 0.01$), Na^+ ($P < 0.001$) in the disease control group when compared to the normal control group. However K^+ ($P < 0.001$), Cl^- ($P < 0.01$) and HCO_3^- ($P < 0.001$) demonstrated an observed increase. Intoxication of experimental rats with valproic acid induced kidney damage and electrolyte imbalance revealed by elevation of UA (116%), CR (31%), Na^+ (29%) and depression of K^+ (54%), Cl^- (29%) and HCO_3^- (45%) when compared to the normal control group. Nevertheless sub-acute pretreatment for 30 days with NLS extract (50 mg/kg) protected the rats against valproic acid induced hepatotoxicity as evidence in the reduction of lipid profile and electrolyte biomarkers in the serum. Treatment with 25 mg/kg vinpocetine; 50 mg/kg, 100 mg/kg and 200 mg/kg NLS extract respectively decreased UA (76, 83%, 104%, 68%); CR (81, 65%, 110%, 112%); Na^+ (69%, 108%, 82%, 71%); and increased K^+ (141%, 94%, 110%, 113%); Cl^- (114%, 101%, 77%, 136%) and HCO_3^- (89%, 96%, 97%, 89%). Hepatoprotective activity was dose dependent for only CR and K^+.

Table 7: Effects of valproic acid intoxication and pretreatment with NLS extract on kidney profile and electrolyte of experimental rats following continuous oral sub-acute dosing for 4 weeks

	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6
UA	2.77 ± 0.51	5.97 0.31^{c,d} (116%)	3.30 ± 0.78^{c,e} (83%)	2.65 ± 0.49^{c,e} (104%)	3.80 ± 0.96^{a,e} (68%)	3.55 ± 0.44^{b,e} (76%)
CR	139.3 ± 2.31	182.3 ± 22.03^{b,d} (31%)	154.5 ± 9.09^{a,e} (65%)	135.0 ± 10.0^{c,e} (110%)	134.0 ± 3.61^{c,e} (112%)	147.3 ± 5.56^{b,e} (81%)
Na^+	122.7 ± 4.73	154.7 ± 13.5^{c,d} (26%)	120.3 ± 7.41^{c,e} (108%)	128.5 ± 4.66^{b,e} (82%)	132.0 ± 3.0^{a,e} (71%)	132.5 ± 2.89^{b,e} (69%)
K^+	8.23 ± 0.93	3.77 ± 0.42^{c,d} (54%)	7.97 ± 0.85^{c,e} (94%)	8.68 ± 0.90^{c,e} (110%)	8.80 ± 0.04^{c,e} (113%)	10.08 ± 1.30^{c,e} (141%)
Cl^-	32.67 ± 2.08	23.10 ± 0.79^{b,d} (29%)	32.80 ± 3.63^{b,e} (101%)	30.50 ± 2.08^{a,e} (77%)	36.13 ± 3.87^{c,e} (136%)	34.00 ± 1.41^{c,e} (114%)
HCO_3^-	29.33 ± 1.16	13.10 ± 0.70^{c,d} (45%)	28.86 ± 1.95^{c,e} (96%)	29.00 ± 2.58^{c,e} (97%)	28.00 ± 2.00^{c,e} (89%)	27.50 ± 4.43^{c,e} (89%)

Group 1: Negative control receiving 10 mL/kg b.w. 2% Tween 80; Group 2: Diseases control group receiving 10 mL/kg b.w. 2% Tween 80 + valproic acid 500 mg/kg; Group 3 receiving NLS extract (50 mg / kg b.w.) + valproic acid 500 mg/kg; Group 4 receiving NLS extract (100 mg / kg b.w.) + valproic acid 500 mg/kg; Group 5 receiving NLS extract (200 mg / kg b.w.) + valproic acid 500 mg/kg, Group 6: Reference control receiving vinpocetine (25 mg/kg b.w.) + valproic acid 500 mg/kg. NLS = *Nauclea latifolia* stem-bark; UA: uric acid; CR: creatinine; Na: sodium; K: potassium; Cl-: chloride; HCO_3^-: bicarbonate. Values presented as mean ± standard deviation ($n = 3 – 7$); $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ ^{d}Values are compared with the negative control group, ^{e}Values are compared with the diseases control group; all values were compared using one way ANOVA and Turkey Test.

3.8 Liver, kidney and lungs histopathology

The histological evaluation of the liver, kidney and lungs were evaluated by examination of the gross macroscopic and microscopic anatomical features. On the liver hispathological evaluation shows that group 1, the negative control, demonstrate a normal liver tissue displaying a central vein, hepatocytes and

sinusoids consistent with normal histology; group 2, the disease control group, shows a liver section with massive recruitment of inflammatory cells consistent with centrolobular necrosis and liver injury; group 3, 4, and 5, the test control groups, all showed moderate, mild and milder depletion of inflammatory cells suggestive of increasing degree of healing process in the hepatocytes; group 6 revealed areas of necrosis. The result posited that the histomorphological injuries induced by VPA on the liver cytoarchitecture by VPA were altered by subacute administration of the medium and higher concentrations of NLS extract thereby offering hepatoprotection.

(Figure 1).

In the kidney (Figure 2) shows normal glomerulus with intact Bowman's capsule, renal tubules with a rich columnar epithelium consistent with normal histology; Group 2 shows a kidney with massive recruitment of inflammatory cell consistent glomerulus nephritis; group 3, 4 and 5 show normal histology as in group; group 6 reveal likely interaction between the valproic acid and standard drug resulting tubular atrophy. The result specify that histomorphological injury induced by VPA on the kidney cytoarchitecture by VPA was significantly altered with aubacute NLS extract demonstrating renoprotection.

On the histopathology of the lungs, group 1 the negative control group showed normal lungs with the respiratory portion with patent alveoli, the alveoli with a normal interstitial tissue, alveolar sac and epithelium consistent with normal histology. Group 2 (disease control group) shows pulmonary oedema, peri-bronchial inflammation, diffuse alveolar damage with numerous infiltrations of inflammatory cells, congested vessels (bronchopneumonia and interstitial pneumonitis. Group 3, 4 and 5 (test groups) exhibited: alveolar spaces permeation of mixed inflammatory infiltrates, alveolar spaces permeation by chronic inflammatory infiltrates with congested blood vessels, alveolar spaces infiltrated by heavy chronic inflammatory infiltrates with mild degree of fibrosis respectively pathologies suggestive of lobar pneumonia. Group 6 (reference control) shows alveolar spaces infiltrated by chronic inflammatory

infiltrates with mild but increased degree of fibrosis and few congested blood vessels (lobar pneumonia). The result indicated that histomorphological injury induced by VPA on the lungs cytoarchitecture was not altered either by subacute administration of NLS extract or standard drug.

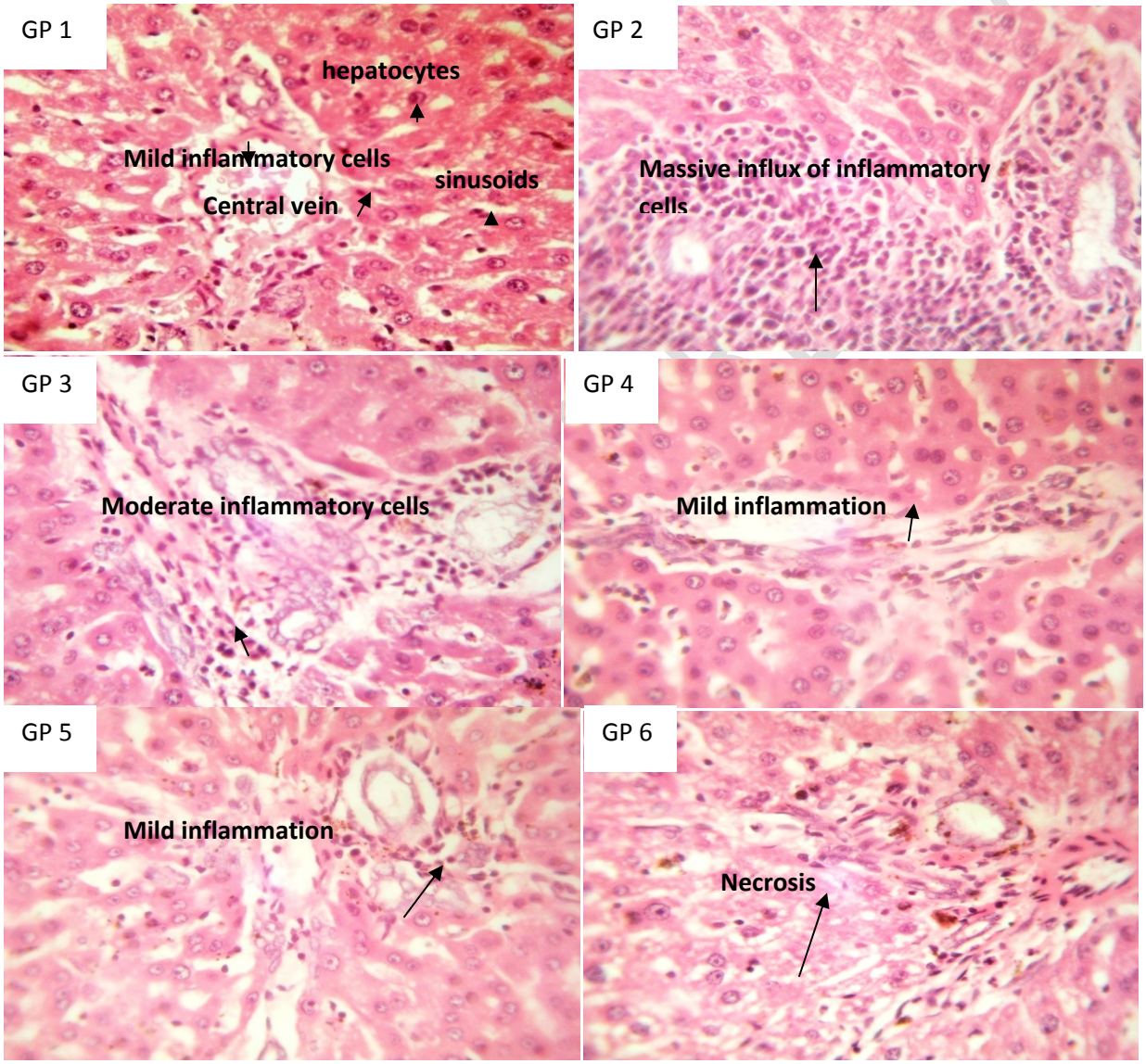

Fig 1. Photomicrograph of heamatoxylin and eosin stained liver sections x400 magnification.

Group 1 (Negative control) receiving 10 mL/kg b.w. 2% Tween 80 shows a normal liver tissue displaying a central vein, hepatocytes and sinusoids consistent with normal histology. Group 2 (disease control group receiving 10 mL/kg b.w. 2% Tween 80 followed by valproic acid 500 mg/kg shows a liver section

with massive recruitment of inflammatory cell consistent centrolobular necrosis and liver injury. Group 3, 4, and 5 (test control groups) received 50, 100 and 200 mg/kg b.w. NLS extract, for each + 500 mg/kg b.w. valproic acid kg/kg b.w all showed moderate, mild and milder depletion of inflammatory cells while Group 6 revealed areas of necrosis. Valproic acid induces liver damage while medium and higher concentration of extract exhibited hepatoprotection. NLS = *Nauclea latifolia* stem-bark extract.

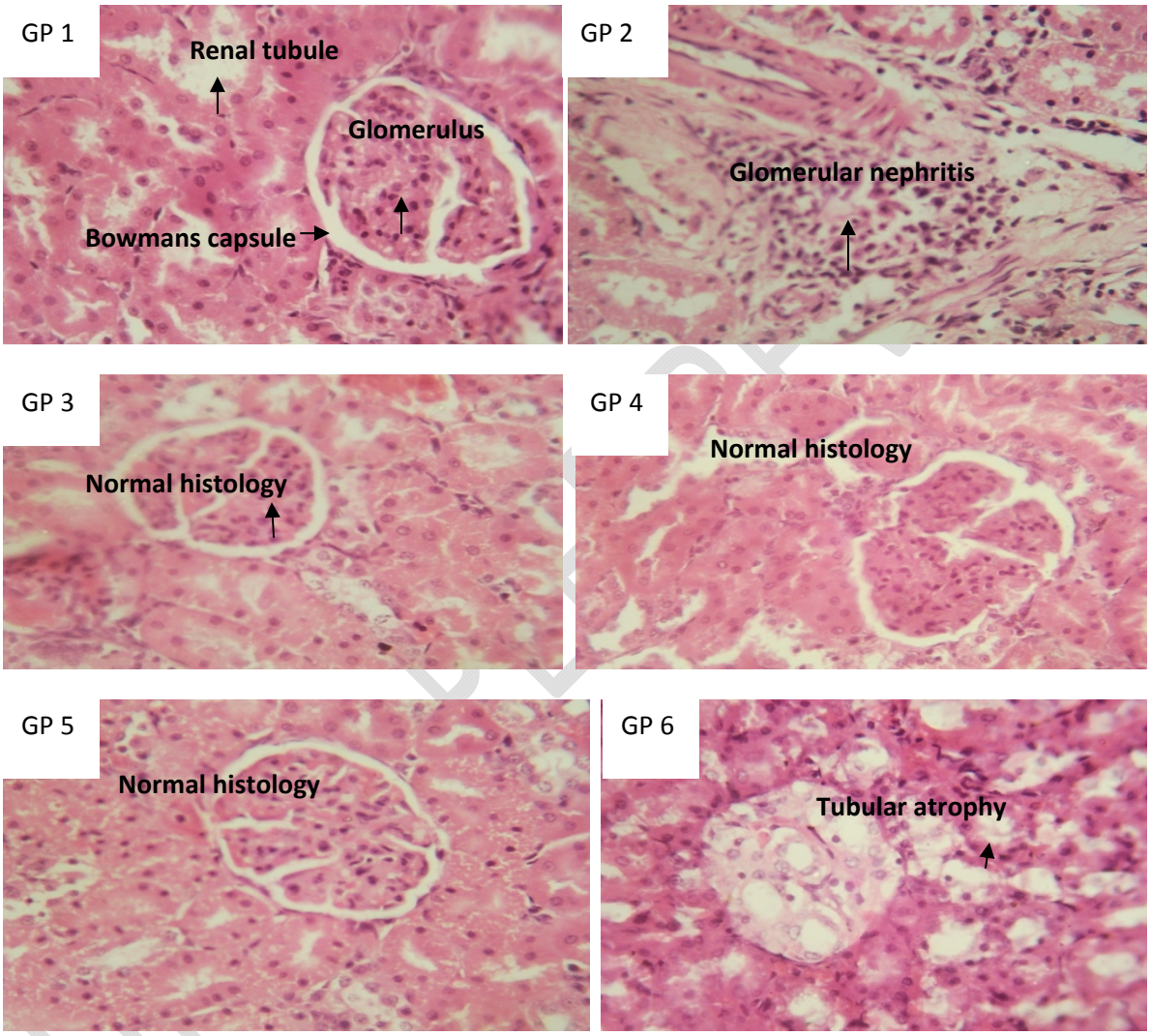

Fig 2. **Photomicrograph of heamatoxylin and eosin stained Kidney sections x400 magnification**.

Group 1 (Negative control) receiving 10 mL/kg b.w. 2% Tween 80 shows a normal kidney tissue displaying a normal glomerulus showing intact Bowman's capsule, renal tubules with a rich columnar epithelium consistent with normal histology. Group 2 (disease control group receiving 10 mL/kg b.w. 2% Tween 80 followed by valproic acid 500 mg/kg shows a kidney with massive recruitment of inflammatory cell consistent glomerulus nephritis. Group 3 , 4, 5 (test control groups) received 50, 100 and 200 mg/kg b.w. NLS extract for each + 500 mg/kg b.w. valproic acid kg/kg b.w all showed renal

tissue consistent with normal histology as shown in group 1. Gp 6 Group 6 (reference control receiving 25 mg/kg b.w. vinpocetin followed by 500 mg/kg b.w. valproic acid shows interaction between the valproic acid and standard drug resulting tubular atrophy. Valproic acid is injurious to the kidney while extract is renal protective. NLS = *Nauclea latifolia* stem-bark extract.

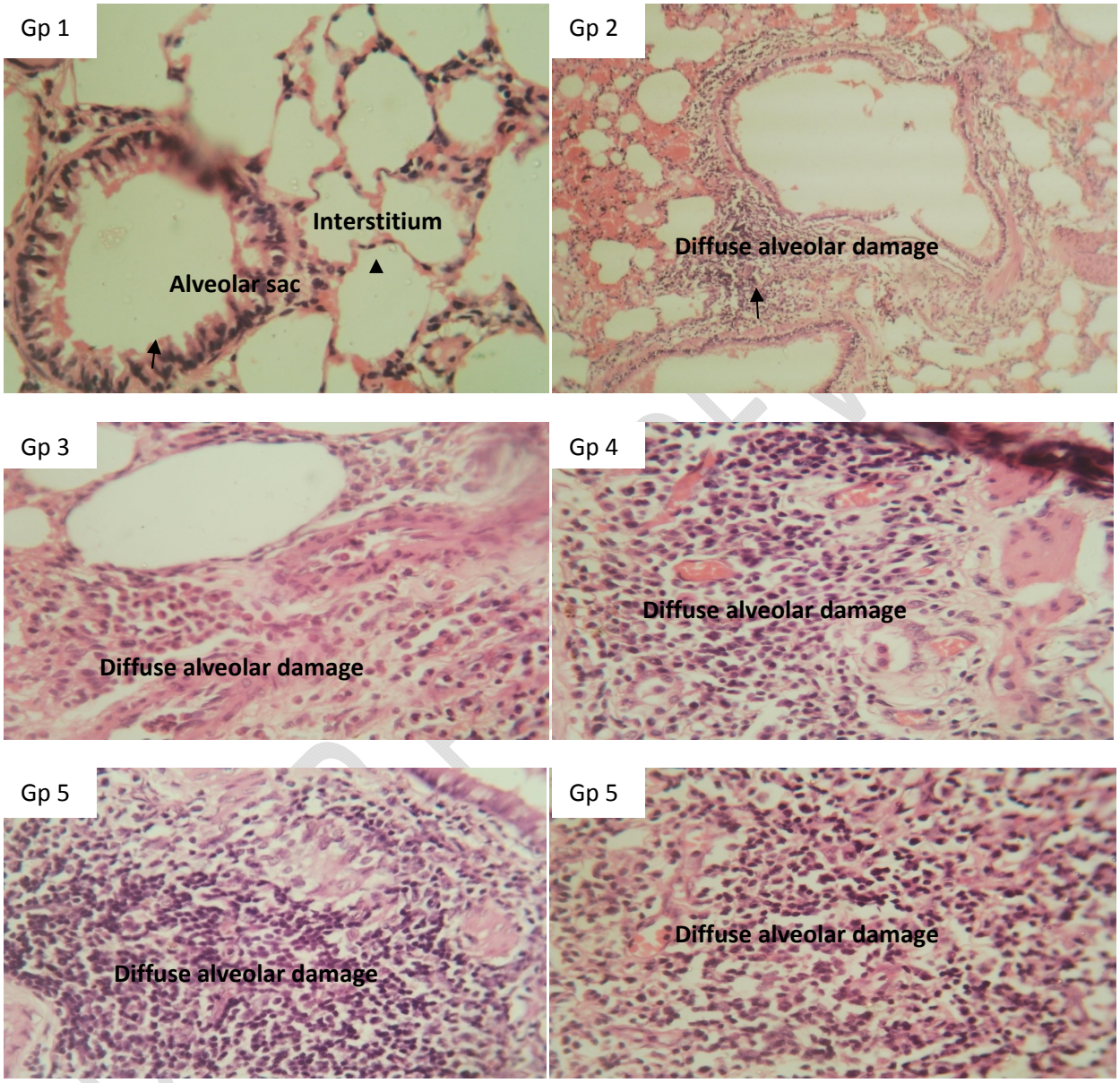

Fig 3. **Photomicrograph of heamatoxylin and eosin stained lung sections x400 magnification**. Group 1 Negative control receiving 10 mL/kg b.w. 2% Tween 80 shows along tissue showing the respiratory portion, the alveoli with a normal interstitial tissue, alveolar sac and epithelium consistent with normal histology. Group 2 (disease control group receiving 10 mL/kg b.w. 2% Tween 80 followed by valproic acid 500 mg/kg shows diffuse alveolar damage with numerous inflammatory cells. Group 3,4 and 5 (test control groups) received 50, 100 and 2000 mg/kg b.w. NLS extract for each + 500 mg/kg b.w. valproic acid kg/kg b.w. exhibited the same pathology seen in group 2. Extract is not protective. Group 6 (reference control) receiving 25 mg/kg b.w. vinpocetin followed by 500 mg/kg b.w.

valproic acid demonstrated the same pathology seen in group 2. The NLS extract and standard drug is not protective too.

4. DISCUSSION

Valproic acid, (VPA) is significant in antiepileptic therapy and is also widely used as a mood stabilizer in biopolar mania [17, 18]. The potential mechanism of action is probably by inhibition of Gamma Amino Butyric Acid (GABA), ion channels and Histone deacetylase [51]. VPA has been linked with numerous toxicities most serious being hepatotoxicity, neurotoxicity and teratogenicity [52]. Hepatotoxicity has been associated with formation of toxic metabolites, (E)-2,4–diene VPA and two conjugation forms with glutathione as N-acetylcystein (NAC) conjugation, NACI and NAC 2 or by medication of lipid peroxidation [51, 52].

Drug-induced toxicity is one of the most commonly encountered difficulties in medicine. Sodium valproate (VPA) is among many drugs with reported pan toxicity effects. The dose makes the poison or *"Sola dosis facit venenum";* this maxim applies to VPA, as experimental toxicity dosage range in animals is in excess of 400 - 750 mg /kg [53, 54, 55] much more than dose use in therapy but chronic dosing of therapeutics doses have manifested broad range of toxicities (7 -16). The potential of NLS ameliorating or abrogating these VPA –induced toxicities is being examined.

In the present study, there was steady weight gain ($P < 0.001$) during the treatment period. This indicates the absence of weight related adverse effect and possible improvement in nutritional status [30]. Previous study by Kouadio et al. [30], also demonstrated significant weight gain in rats administered the aqueous stem -bark extract of *Nauclea latifolia.* Besides, no specific target organ toxicity was observed in rats intoxicated with VPA and those treated with NLS extract; but significant reduction in the liver weight of animals administered VPA 500 mg/kg and NLS extract 50 mg/kg body weight ($P < 0.05$) was noted. The doses of NLS extract utilized demonstrated considerable potential in alteration of histomorphological insult induced by VPA on the liver cyto architecture thereby mediating a hepatoprotective effects.

The treatment and intoxication showed no statistically significant difference on hematological indices across all groups. Probably this might be due to high rate of recovery of blood cells from the effect of VPA. This do not corroborate the study of Kouadio et al. [30] who reported elevation of platelets, erythrocytes and eosinophils reflecting potential benefits in blood coagulation, anemia and allergenic effect respectively . Valproic acid in previous studies has been shown to cause coagulopathy, platelet

dysfunction and thrombocytopenia thought to be associated with possibly direct toxicity on bone marrow or VPA inclusion into platelet membrane due to similar fatty acid cell membranes [51].

The liver function enzymes [Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), Aspartate Amino Transferase (AST), Gamma Glutamyl Transferase (GGT)] serum activities are used as biomarkers of liver damage as leakage of these enzymes into blood stream is associated with hepatocyte injury [56]. A very sensitive indicator of insult to hepatocyte is the release of liver enzymes such as AST and ALT after VPA intoxication [52], the elevated activities as seen in this study indicated hepatocellular damage which concur with the study by Morsy et al. [7]. AST is distributed in the body tissues; including muscle, heart and liver while ALP in the liver, kidney and bone. In the liver, AST is mainly present in the mitochondria of hepatocytes while ALT is found outside the mitochondria. Treatment with NLS extract promoted significant decrease in liver enzymes ($P < 0.05$ - 0.001). The reduction in GGT by NLS treatment was dose dependent progressing from 85 - 111% with the increasing doses of extract (50 – 200 mg/kg body weight). There was also reduction in AST and ALT and this agree with previous studies that demonstrated *Nauclea latifolia* hepatoprotective effects in down-regulating serum liver enzymes concentration [30]. Though present results is not dose dependent but corroborate the reduction in liver function enzymes report of Kouadio et al.[30] (2014) in sex and time dependent manner. However the effects on liver enzymes reported the present investigation do not corroborate earlier report of Arise et al. [32] that prolong administration of NLS extract posit grave danger as a surge in liver function enzymes were observed in the serum, liver and kidney homogenates from subacute dosing in a dose and time dependent fashion in their report; besides their report also revealed increased in the propensity of disruptions in amino acid catabolism, liver and kidney damage as conclusion of their investigation.

Valproic acid has been known to cause protein depletion and hypoalbuminema from previous studies. This was also demonstrated in this study. This effect of VPA was countered in a dose dependent manner by elevation of serum protein and albumin in rats treated with NLS extract. Bilirubin is a product of haemoglobin breakdown. Its serum level is important for assessing liver function and an irregular increase

in bilirubin level indicates hepatobiliary disease or hepatobiliary damage and conjugated hyper bilirubinaemia. This protein depletion was attenuated non-dose dependently indicating higher hepatoprotective activity as the percentage hepatoprotection increased from of 108% to 153% following increased dose from 50 to 100 mg/kg of NLS extract respectively in the rat.

Cholesterol tends to undertake inactive swapping between plasma lipoproteins and cell membranes leading to abnormal cholesterol deposition [57]. As seen in this study, other studies have demonstrated the effect of VPA intoxication in significantly increasing lipid profile: total cholesterol, triglycerides, phospholipids and free fatty acids mainly. Treatment with NLS extract showed a decrease in the total cholesterol, triglycerides, low density lipoprotein, and very low density lipoproteins elevated by Valproic acid intoxication. However, valproic acid decreased the levels of high density lipoprotein cholesterol which was significantly elevated with the treatment of the NLS extract depicting attenuation or antagonism of VPA -induced hypolipidermia. Uric acid has been implicated in numerous chronic diseases such as hypertension, metabolic syndrome, diabetes, non-alcoholic fatty liver disease, and chronic kidney disease [58]. Uric acid is implicated in steatosis of liver by mitochondria damage. Increase in lipid concentration e.g. triglycerides can also be correlated with increased serum uric acid level [59]. The disease control group demonstrated nephrotoxicity percentage of 116% following intoxication with VPA indicating hyperuricaemia suggestive of synergism of both diuretic potentials of NLL with VPA-mediated toxicity profile. This was effectively attenuated by administration of NLS extract. Creatinine is a breakdown product of muscle tissues, rapid increases in serum creatinine can be associated with renal damage. Previous study by Kouadio et al. [30] showed a significant decrease in serum creatinine with *Nauclea latifolia* stem bark aqueous extract. This concurs with our report as NLS extract enhanced the reduction of Valproic acid-elevated creatinine level. Serum creatinine was reduced dose dependently in the NLS extract treated groups.

Effect of valproic acid intoxication on electrolyte was associated with increased in the level of Na^+, and decreased in the levels of K^+, Cl^- and HCO_3^-, which was reversed by co-administration with the NLS extract treatment groups. Both NLS and VPA is reported to exhibit diuretic effect [30, 60]. The decreased electrolytes observed are consistent with the diuretic effects of NLS extract.

The biochemical effects observed corroborated the histopathological study on the liver and the kidney to mediate hepatoprotection and nephroprotection respectively. However, no protection was observed on lung cytoarchtecture. Kouadio et al. [30] reported no-observable-adverse-effect-level of NLS aqueous extract range from 1.8 – 18 mg/kg and reported elevated inflammatory potentials likely due to high

eosinophilia. Our study doses range of 50 – 200 mg/kg might in part likely aggravate VPA-induced toxicity. Worst still, Arise et al. [32] reported liver dysfunction and impingement in the secretory and excretory functions of the kidney due to downregulation of total cholesterol and urea following subacute administration of NLS extract for 28 days. The histopathological results of this study corroborated the biochemical report of Arise et al. [32].

The reference drugs vinpocetine utilized in this study posit possibility of potential drug-drug interaction and or bioavailability concerns as it was unresponsive to the VPA-induced toxicological insults across all the tissues, liver, kidney and lungs in the administered posology. In rats, oral bioavailability of vinpocetine of 52% have been reported suggesting extensive first pass metabolism following oral administration [61, 62].

The bioactive agents present in NLS hydromethanolic extract analyzed include moderate abundance of saponin, cardiac glycosides and slight abundance of flavonoids, tannins, and phlobatannins. These phytochemicals and bioactive moiety which might in part contribute significant impact on the observed pharmacological and toxicological effects of NLS extract in this study.

5. CONCLUSION

The findings of this study revealed that *Nauclea latifolia* stem-bark ameliorated the histomorphological injury induced by VPA on the liver, kidney but not on the lungs cytoarchitecture. The study posits that the potential VPA- mediated disruption of the liver and kidney cytoarchitecture could in part be reversed with NLS extract administration in the liver and kidney thus demonstrating hepato-and nehro-protective propensity. This study gave credence to positive or beneficial therapeutic effect of NLS extract. Our investigation support safety on subacute dosing and gave credence to ethnomedicinal patronage for primary health care utility of NLS extract infusions and decoctions for the treatment of stomach pain, constipation, fever, and diarrhea.

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