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Research Article

# Subchronic Study of *Nauclea latifolia* Leaf Extract on Valproic Acid-Induced Toxicity in Liver, Lung and Kidney of Rats

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## Abstract

**Objectives:** This study was conducted to examine the sub-chronic toxicity effects of a Histone deacetylase (HDAC) inhibitor, sodium valproate or valproic acid (VPA) (Epilim®), in rats and the possible protective effect of *Nauclea latifolia* leaf (NLL) hydromethanolic extract.

**Materials and Methods:** Treated normal control rats received distil water (10 mL/kg), the diseases control group received VPA (500 mg $kg^{-1}$ $d^{-1}$), the experimental groups received NLL at doses of 100, 200, 400 mg $kg^{-1}$ $d^{-1}$ plus VPA 500 mg $kg^{-1}$ $d^{-1}$ respectively and the reference group received 25 mg $kg^{-1}$ $d^{-1}$ of vinpocetine plus VPA 500 mg $kg^{-1}$ $d^{-1}$ for 4 weeks. Hematological and biochemical analysis were performed. Valproic acid intoxication-induced hepatotoxicity in the liver, alteration in lipid and electrolytes profiles and kidney functions evidenced by elevated levels of biomarkers of toxicity were evaluated. Histopathological alterations in liver, kidney and lungs were evaluated.

**Results:** Serum total protein was significantly reduced while liver function enzymes were significantly upregulated. Significant elevation of total bilirubin and conjugated bilirubin in VPA-exposed rats suggestive of hepatic and biliary dysfunction respectively were observed. Valproic acid produced several histopathological changes in liver and lungs but not in the kidney. The present study affirms histopathology-related biochemical alterations notably in liver and kidney but not the lungs as potential biomarkers for VPA -induced toxicity in rats. Co-administration of NLL produced considerable protection at the biochemical and at the histological levels of both liver and kidney but not the lungs.

**Conclusion:** *Nauclea latifolia* hydromethanolic leaf extract is of potential value in ameliorating the toxic insult produced by VPA at biochemical but not the cytoarchitecture of rats.

***Keywords:*** *Sub-Chronic Toxicity; Sodium Valproic Acid; Hepatotoxicity; Histopathology; Nauclea latifolia*

## Background and Case Report

Drug-induced toxicity or injury cut-across a spectrum of pathologies, some of which are related to the mode of injury or the cell type primarily damaged [1]. Besides these, drug-induced injury is characterized by the alteration and or destruction witnessed at the biochemical and the histological architectures following exposure to a drug(s) or chemical intoxication [2,3]. Toxicity or injury is an indication of the degree to which a substance is poisonous or can induce injury. Toxicity is multi-factor's dependent with the dose of the poison, duration of exposure to the poison and the chemistry of the agents entwining its complexity. Adverse effects may be in single or multiple

organs with potential threat to such an organ and the life of the organism [3]. Prospective toxicity targets and potential impingement are the neuron (neurotoxicity), the liver (hepatotoxicity), the blood (hematotoxicity), the kidney (nephrotoxicity), the reproductive system (reproductive toxicity), the immune system (immunotoxicity) and the gene (genotoxicity) etc. Toxicological alteration in the structural and or functional integrity of the system might lead to an adverse effect which might be permanent or irreversible damage. An adverse effect is any treatment related effects which might interfere with the chemistry, structure or function of the cells, tissue or system, during development, or at maturity [4]. External and internal agents have been incriminated in systemic toxicity. Accumulation of aberrant proteins (beta amyloids), neurotransmitters (glutamate, dopamine), redox mediated posttranslational proteins modifications, oxygen and nitrogen radicals (oxidative or nitrosative stress-induced redox reactions), inflammatory mediators are reported to underpin toxicity in several milieu. Many aberrant proteins play crucial role in the pathogenesis of many neurodegenerative diseases such as Alzheimer disease. Toxicological impingement on the hepatic or cardiovascular structures and or an interference with the endocrine system might in part lead to multi-focal damage or alteration to the physiology or histology of the tissues.

Depending on the dose, most drugs are safe or therapeutic but at high dose are toxic thus affirming the maxim of Paracelsus, the father of toxicology that "poison makes the dose". Increasing evidence reveal that valproic acid (VPA) is neuroprotective at low doses [5] and toxic at high dose. Multi-focal systemic VPA-induced toxicity reported are neurotoxicity [6], hepatotoxicity [7], hematotoxicity [8], nephrotoxicity [9], pancreatitis [10], bone marrow suppression [11], teratogenicity and developmental toxicity [12,13] and numerous idiopathic effects which in the offspring might lead to autistic spectrum disorder [14]. Valproic acid is effective to treat or prevent epilepsy, migraine and bipolar disorders [15]. The mechanism of valproic acid induced neurotoxicity is by VPA-induced hyperammonemic encephalopathy (VHE) [16]. Acute toxicity studies show valproate to possess an oral $LD_{50}$ ranging from 1100 to 3900 mg/kg in rodents [17].

Utilization of phyto-therapy in the management of multi-organ toxicity is uncommon in African traditional medicine. Reports of amelioration of specific target organ induced toxicity such as neurotoxicity [18], hepatotoxicity [19], hematotoxicity [20], nephrotoxicity [21], reproductive toxicity [22], with different plant extract is ubiquitous in literature.

The amelioration of toxicity is most often linked to bioactive agents (flavonoids, saponins, polyphenols, etc.) in plants phytochemicals. Flavonoids for example exhibits a wide range of biological activities such as antimicrobial, anti-inflammatory, antiangiogenic, analgesic, antiallergic effects, cytostatic, and antioxidant properties [23]. Aliyu., *et al*. [24] reported that phenolic compounds are the major group of compounds that acts as primary antioxidant because they mop-up oxygen free radicals, hydroxyl, superoxide anion radicals and lipid peroxyl radicals [24]. Though a high correlation between antioxidant activity and phenolic compounds is reported but at certain doses they may be toxic due to their pro-oxidant and oxidant effects.

This study seeks to investigate the effectiveness of NLL to abrogate multifocal toxicity induced by VPA on the biochemical profiles and possible protective effects on the liver, lungs and kidney cyto-architectures.

*Nauclea latifolia* (Rubiaceae) is an evergreen multi-stemmed tree which grows to a height of 200 m. It is generally known as pin cushion tree or as "African peach (English), as mbong-ibon (Ibibios and Effik), as Tafashiya or tafiyayaiga (Ibo), as ubulinu or ovoroilu and Egbe yesi or egbesi (Yoruba) in Nigeria. It is commonly found in the humid tropical rainforest zone or in Savannah woodlands of West and Central Africa. Ethnomedicines has listed wide range of uses some of which have been verified experimentally such as antimalarial [25], anthelminthic [26], antiviral [27], antimicrobial [28], fever [29], diabetes [30], neuropharmacological effects [31]. However, *N. latifolia* leaf extracts effects in abrogating multifocal toxicity induced by valproate acid in rodents is yet to be evaluated hence this investigation.

## Materials and Methods

### Drugs, Chemicals and equipment

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 are: 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).

### Collection and authentication of plant materials

The leaf *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 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.

### Preparation of *N. latifolia* leaf (NLL) extract

*Nauclea latifolia* dried leaf (NLL) was pulverized to fine particles using mechanical grinder. A 250 g leaf powder was macerated in 2 litres of *n*-hexane to defat it for 24 hours, after which the extract was concentrated using a rotary evaporator and the residue soaked in 2 liters of methanol for 72 hours; while shaking vigorously every 2 hour for 12 hours. The extract was concentrated using rotary evaporator, before drying on the water bath at 45°C. The percentage yield was estimated.

### Phytochemical screening

Phytochemical screening of the plant extract was executed at the Pharmacognosy and Phytotherapy Department laboratory of the University of Port Harcourt. The bioactive agents screened include: flavonoids, triterpenoids, saponins, cardiac glycosides, alkaloids, and phlobatannins using standard protocol [32].

### Animals

The experimental animals are forty male Wistar albino rats weighing about 150 - 170 g 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 unconstrained 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).

### Acute toxicity study

The acute toxicity of the hydro-methanol leaf extract of *Nauclea latifolia* already established by Morufu., *et al*. [33] and was determined to be 1600 mg/kg. In our study we choose doses of 100, 200 and 400 mg/kg for the investigation.

### 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 an oral dose 500 mg/kg daily for 4 weeks by gavage in the experimental animals [34]. Each one mL contains 200 mg sodium valproate.

The animals were divided into six groups with 6 animals per group by randomization adopting block permuted plan. The NLL, valproate and vinpocetine (Cognitol®) (the reference drug) were administered orally per kg of body weight once daily for 4 weeks. 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 NLL and vinpocetine 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 or experimental groups): The animals in these groups received sodium valproate (500 mg/kg) followed by the NLL extract 100 mg/kg, 200 mg/kg and 400 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, NLL or vinpocetine was administered adopting standard procedure [34].

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

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

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

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

### Evaluation of weights animals and relative organs weight

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 abstracted and weighed and the relative organ weights calculated.

### 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 micropipette into vacutainers, stored at - 20$^0$C until used for biochemical analysis.

### 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 [19].

### Biochemical serum analysis

Biomarkers of toxicity 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 [35]; alkaline phosphatase (ALP) was reported by the procedure of Roy [36]. Colorimetric assays were used to determined serum total bilirubin (TBIL) and conjugated bilirubin (CBIL) by the methodology of Jendrassik and Grof [37], total protein by Biuret procedure of Flack and Woollen [38], albumin by Doumas., *et al.* [39], high density lipoproteins (HDL) profile by the protocol of Lopes-Virella [40]; total cholesterol (TCHO) was obtained by the procedure of Allain and Roeschlau method [41]; triglycerides (TG) by Burtis and Tietz [42] procedure; and electrolytes such as sodium ($Na^+$) by the procedure of Maruna [43] and Suderman and Delory [44], chloride ($Cl^-$) by the colorimetric procedure of Schoenfeld and Lewellen [44], potassium ($K^+$) and bicarbonate ($HCO_3^{2-}$) by the methodology of Henry., *et al* [45]. Serum creatinine and serum urea were determined by the method of Varley and Alan., *et al* [46].

### Histopathology studies

The tissues, lungs, liver, and kidney harvested from the control and test experimental albino rats were fixed in 10% formal saline. Tissues were sectioned into 4 - 5 µm cross-sections with rotary microtome model Leica RM2125 RT. Staining was optimized with

haematoxylin and eosin for 48 hours using protocol of Kiernan [47] on all slides. Morphological changes were examined in well stained slides under a light microscope after mounting in a mixture of distyrene (polystyrene), plasticizer (tricresyl phosphate) and xylene, generally called DPX mountant (Atom Scientific, Manchester, UK). Kidney tissue specimen was stained with Grill's modified Hematoxylin and Eosin was performed and representative tissue of various organs were collected for standard processing into paraffin-embedded tissue blocks. High power fields (at 400x magnification) was used in the histological observation.

### 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.

## Results

### Phytochemical results

This result shows that the *Nauclea latifolia* hydro-methanol leaf extract contains alkaloids in high quantities, saponins, triterpenoids and cardiac glycosides in moderate quantities; flavonoids and steroids in trace quantities but contained no tannins (Table 1).

| Constituent | Results |
|---|---|
| Alkaloids | +++ |
| Flavonoids | + |
| Saponins | ++ |
| Tannins | - |
| Steroids | + |
| Terpenoids | ++ |
| Cardiac glycosides | ++ |

***Table 1:*** *The phytochemical constituents present or absent in the hydro-methanol extract of Nauclea latifolia leaves.*

*Symbol meanings: (+) represents presence in trace quantities, (++) represents presence in moderate quantities, (+++) represents presence in high quantities and (-) represents absence of the phytochemical constituents.*

### Effect on weights

This result shows that all groups had a general increase in weight. Groups 4 and 5 showed significant (*P* < 0.001) weight increase in week 1 when compared to group 2. Group 6 showed a significant (*P* < 0.01) increase in weight on the third week when compared to the initial weights. Groups 4 and 6 also showed significant (*P* < 0.05) and (*P* < 0.01) respective increase in initial weights on the fourth week with only Group 6 showing a significant (*P* < 0.05) increase in initial weight with respect to the final weight. This effect is shown in table 2.

| Weights (g) | | | | | | |
|---|---|---|---|---|---|---|
| Group | Initial Weight | Week 1 | Week2 | Week3 | Week4 | Final Weight |
| Group 1 | 162.6 ± 23.7 | 164.8 ± 23.7 | 167.3 ± 25.1 | 167.9 ± 18.7 | 183.6 ± 30.45 | 167.9 ± 18.7 |
| Group 2 | 168.3 ± 6.3 | 175.8 ± 2.8 | 182.8 ± 10.3 | 184.4 ± 17.6 | 178.1 ± 38.3 | 184.4 ± 17.6 |
| Group 3 | 174.5 ± 26.1 | 172.4 ± 36.7 | 180.1 ± 27.2 | 177.7 ± 34.2 | 176.1 ± 32.8 | 177.7 ± 34.2 |
| Group 4 | 143 ± 19.2 | 150.4 ± 15.4$^{c,e}$ | 160.7 ± 13.0 | 170.6 ± 13.7 | 180.4 ± 14.5$^{a,f}$ | 170.6 ± 13.7 |
| Group 5 | 153.5 ± 37.6 | 156.4 ± 38.8$^{c,e}$ | 159.7 ± 33.2 | 155.6 ± 32.6 | 164.7 ± 26.9 | 155.6 ± 12.6 |
| Group 6 | 145.7 ± 6.8 | 154.3 ± 9.7 | 172.8 ± 17.8 | 185.2 ± 16.4$^{b,f}$ | 190.5 ± 17.4$^{b,f}$ | 185.2 ± 16.4$^{a}$f |

***Table 2:*** *Effects of valproic acid intoxication and post-treatment with NLL hydro-methanol extract on body weights of experimental rats following continuous oral sub-chronic dosing for 4weeks.*

*Group 1: Negative control group 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 NLL extract (100 mg/kg b.w.) ± valproic acid 500 mg/kg; Group 4 receiving NLL extract (200 mg/kg b.w.) ± valproic acid 500 mg/kg; Group 5 receiving NLL extract (400 mg/kg b.w.) ± valproic acid 500 mg/kg, Group 6: Reference control receiving Vinpocetine (25 mg/kg b.w.) ± valproic acid 500 ml/kg. NLL: Nauclea latifolia leaf. Values presented as mean ± standard deviation (n = 3 - 5); $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, $^{e}$values are compared with Group 2, $^{f}$values are compared with initial weight using one way ANOVA and Turkey Test.*

### Relative organ weights

The effects of NLL hydro-methanol extract on the relative organ weights are summarized in table 3. This table shows that there was no statistically significant change in the relative weights of the organs analyzed when compared to the disease control group.

| Organs | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 |
|---|---|---|---|---|---|---|
| Liver | 4.08 ± 0.67 | 4.86 ± 1.20 | 4.63 ± 0.79 | 4.40 ± 0.30 | 4.08 ± 0.34 | 4.03 ± 0.35 |
| Kidney | 0.61 ± 0.07 | 0.74 ± 0.23 | 0.69 ± 0.09 | 0.64 ± 0.06 | 0.63 ± 0.07 | 0.75 ± 0.05 |
| Lungs | 1.07 ± 0.27 | 1.23 ± 0.24 | 1.42 ± 0.73 | 1.07 ± 0.13 | 1.09 ± 0.27 | 1.08 ± 0.14 |
| Testes | 1.35 ± 0.20 | 1.95 ± 0.59 | 1.40 ± 0.51 | 1.34 ± 0.16 | 1.43 ± 0.19 | 1.39 ± 0.35 |
| Hearts | 0.36 ± 0.04 | 0.44 ± 0.08 | 0.38 ± 0.03 | 0.35 ± 0.33 | 0.43 ± 0.08 | 0.38 ± 0.04 |
| Brain | 0.85 ± 0.11 | 1.02 ± 0.33 | 0.95 ± 0.09 | 0.94 ± 0.07 | 0.90 ± 0.09 | 0.79 ± 0.11 |
| Stomach | 1.99 ± 0.58 | 1.61 ± 0.59 | 2.11 ± 0.89 | 1.62 ± 0.37 | 1.44 ± 0.13 | 1.24 ± 0.19 |
| Spleen | 0.48 ± 0.16 | 0.58 ± 0.24 | 0.54 ± 0.16 | 0.46 ± 0.15 | 0.44 ± 0.11 | 0.39 ± 0.17 |

***Table 3:*** *The effect of valproic acid intoxication and post-treatment with NLL hydro-methanol extract on relative organ weights following continuous oral sub-chronic dosing for 4 weeks.*

*Group 1: Negative control group 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 ml/kg; Group 3 receiving NLL extract (100 mg/kg b.w.) + valproic acid 500 ml/kg; Group 4 receiving NLL extract (200 mg/kg b.w.) + valproic acid 500 mg/kg; Group 5 receiving NLL extract (400 mg/kg b.w.) + valproic acid 500 mg/kg, Group 6: Reference control receiving vinpocetine (25 mg/kg b.w.) + valproic acid 500 ml/kg. NLL = Nauclea latifolia leaf. Values presented as mean ± standard deviation (n = 3- 5). Relative organ weight was calculated as (organ weight (g)/body weight of animal on sacrifice day (g) × 100.*

### Effects on hematological indices

The effects of NLL extract on the hematological indices are summarized in table 4. This result shows that there was no statistically significant change in the hematological indices of the albino rats analyzed when compared to the disease control group.

| Parameters | Unit | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 |
|---|---|---|---|---|---|---|---|
| RBC | $10^{12}$/L | 5.86 ± 0.86 | 5.28 ± 0.74 | 4.85 ± 0.31 | 5.50 ± 0.75 | 5.53 ± 1.01 | 5.2 ± 0.66 |
| HGB | g/dL | 12.14 ± 0.83 | 11.5 ± 1.15 | 10.83 ± 0.31 | 11.85 ± 1.01 | 11.5 ± 1.16 | 11.1 ± 1.02 |
| WBC | $10^{9}$/L | 15.48 ± 2.68 | 13.88 ± 5.69 | 17.45 ± 3.31 | 18.63 ± 10.55 | 9.0 ± 3.39 | 10.9 ± 3.61 |
| PCV | (%) | 36.4 ± 2.51 | 34.5 ± 3.42 | 32.5 ± 1.92 | 35.5 ± 3.00 | 34.5 ± 4.44 | 33.3 ± 3.06 |
| MCV | fL | 62.64 ± 4.47 | 65.73 ± 3.90 | 67.03 ± 1.26 | 64.93 ± 3.80 | 63.23 ± 5.11 | 64.33 ± 2.30 |
| MCH | Pg | 20.92 ± 1.52 | 21.9 ± 1.24 | 22.33 ± 0.49 | 21.75 ± 1.15 | 21.08 ± 1.75 | 21.4 ± 0.76 |
| MCHC | g/dL | 33.3 ± 0.08 | 33.3 ± 0.08 | 33.28 ± 0.10 | 33.35 ± 0.10 | 33.33 ± 0.05 | 33.27 ± 0.06 |
| PLT | $10^{9}$/L | 242 ± 38.7 | 309 ± 40.52 | 280 ± 29.0 | 263.5 ± 60.4 | 333 ± 15.9 | 289.7 ± 27.3 |
| MPV | Fl | 8.6 ± 0.35 | 8.75 ± 0.54 | 8.68 ± 0.59 | 8.35 ± 0.29 | 8.53 ± 0.29 | 8.30 ± 0.27 |
| LYM | (%) | 58.6 ± 2.61 | 63.0 ± 6.27 | 57.8 ± 5.12 | 65.0 ± 7.02 | 62.3 ± 2.06 | 65.7 ± 8.15 |
| NEUT | (%) | 26.6 ± 2.70 | 25.0 ± 4.76 | 27.3 ± 4.11 | 21.8 ± 4.03 | 26.8 ± 1.50 | 25.7 ± 5.13 |
| EOSINO | (%) | 4.00 ± 2.00 | 3.25 ± 0.96 | 4.00 ± 0.82 | 3.50 ± 1.00 | 3.25 ± 1.26 | 2.67 ± 1.53 |
| MONO | (%) | 10.8 ± 0.84 | 8.25 ± 2.75 | 10.75 ± 1.26 | 9.75 ± 3.40 | 7.75 ± 0.96 | 6.00 ± 2.65 |

***Table 4:*** *The effect of valproic acid intoxication and post-treatment with NLL hydro-methanol extract on hematological parameters following continuous oral sub-chronic dosing for 4 weeks.*

*Group 1: Negative control group 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 NLL extract (100 mg/kg b.w.) + valproic acid 500 ml/kg; Group 4 receiving NLL extract (200 mg/kg b.w.) + valproic acid 500 mg/kg; Group 5 receiving NLL extract (400 mg/kg b.w.) + valproic acid 500 ml/kg, Group 6: Reference control receiving vinpocetine (25 mg/kg b.w.) + valproic acid 500 ml/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; NLL: Nauclea latifolia Leaf Values Presented as mean ± SD (n = 3- 5). One -way Analysis of variance (ANOVA) followed by post hoc Turkey's multiple comparison Test.*

### Effects on liver enzymes

The effects of NLL extract following sub-chronic intoxication with valproic acid on liver enzymes are summarized in table 5. The result indicates statistically significant increase in GGT ($P < 0.01$), AST ($P < 0.01$). TP ($P < 0.01$), TBIL ($P < 0.05$) and CBIL ($P < 0.05$) and non-significant increase in ALP in the diseases control group when compared to the negative control group. Intoxication of experimental rats with valproic acid induced hepato-cellular damage revealed by significant elevation ($P < 0.05$ - 0.001) in the levels of serum GGT (71%), AST (38%), ALT (84%) TP (28%), TBIL (27%) and CBIL (41%) and non-significant increase ALP (12%), compared to the negative control group. Nevertheless, sub-chronic post-treatment for four weeks with NLL extract protected the rats against valproic acid- induced hepatotoxicity as evidenced by the reduction of hepatic damage biomarkers in the serum. Post-treatment with NLL 100 mg/kg body weight reduces GGT (88%), AST (190%), ALT (103%), ALP (71%), T.P (104%), TBIL (124%) and CBIL (77%). Similarly, post-treatment of NLL 200 mg/kg reduces GGT, AST, ALT, ALP, T.P, TBIL, CBIL by 128%, 180%, 113%, 120%, 133%, 146%,104% while post-treatment of NLL 400 mg/kg reduces these indicators by 81%, 207%, 118%, 143%, 145%, 111%, 44% respectively and reference drug, vinpocetine reduces these indicators by 149%, 159%, 115%, 134%, 157%, 153%, 20% respectively compared to the disease control group. The hepato-protective activity was dose dependent for ALT alone.

| Parameters | GRP 1 | GRP 2 | GRP3 | GRP4 | GRP5 | GRP6 |
|---|---|---|---|---|---|---|
| GGT | 1.66 ± 0.38 | 2.84 ± 0.60$^{\#\#}$ (71%) | 1.80 ± 0.27** (88%) | 1.33 ± 0.36*** (128%) | 1.88 ± 0.05* (81%) | 1.08 ± 0.20*** (149%) |
| AST | 62.60 ± 7.23 | 86.25 ± 4.35$^{\#\#}$ (38%) | 41.25 ± 9.95*** (190%) | 43.75 ± 10.69*** (180%) | 37.25 ± 5.74*** (207%) | 48.67 ± 10.41*** (159%) |
| ALT | 33.20 ± 1.48 | 61.25 ± 1.71$^{\#\#\#}$ (84%) | 32.25 ± 4.72*** (103%) | 29.50 ± 3.70*** (113%) | 28.00 ± 5.6*** (118%) | 29.00 ± 4.0*** (115%) |
| ALP | 56.60 ± 1.14 | 63.25 ± 3.78 (12%) | 47.50 ± 1.30*** (71%) | 55.25 ± 3.86* (120%) | 53.75 ± 4.57** (143%) | 54.33 ± 4.04* (134%) |
| T.P | 62.80 ± 1.92 | 45.00 ± 2.31$^{\#\#}$ (28%) | 63.50 ± 3.00** (104%) | 68.75 ± 3.95*** (133%) | 70.75 ± 4.11*** (145%) | 73.00 ± 2.00*** (157%) |
| T.BIL | 20.60 ± 1.34 | 26.25 ± 1.50$^{\#\#}$ (27%) | 19.25 ± 2.22*** (124%) | 15.00 ± 3.74*** (146%) | 20.00 ± 1.41** (111%) | 17.67 ± 2.52*** (152%) |
| C.BIL | 9.03 ± 0.46 | 12.75 ± 0.87$^{\#}$ (41%) | 9.90 ± 0.67 (77%) | 8.85 ± 3.21$^{*}$ (104%) | 11.13 ± 1.80 (44%) | 12.07 ± 0.60 (20%) |

***Table 5:*** *Effects of valproic acid intoxication and post-treatment with NLL hydro-methalonic extract on liver enzymes of experimental rats following sub-chronic dosing for four weeks.*

*Group 1: Negative control group 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 NLL extract (100 mg / kg b.w.) +valproic acid 500 mg/kg; Group 4 receiving NLL extract (200 mg / kg b.w.) + valproic acid 500 mg/kg; Group 5 receiving NLL extract (400 mg / kg b.w.) + valproic acid 500 ml/kg, Group 6: Reference control receiving vinpocetine (25 mg/kg b.w.) + valproic acid 500 mg/kg. NLL: Nauclea latifolia Leaf; GGT: Gamma-glutamyl Transpeptidase; ALT: Alanine Transaminase; AST: Aspartate Transaminase; ALP: Alkaline Phosphatase; T.P: Total Protein; T.BIL: Total Bilirubin; C.BIL: Conjugated Bilirubin. Values presented as mean ± standard deviation (n = 3 - 5);* $^{*(\#)}P < 0.05$, $^{**(\#\#)}P < 0.01$, $^{***(\#\#\#)}P < 0.001$. *Analysis was done using one way ANOVA and Turkey Test.*

### Effects on lipid profile

The effect of NLL following sub-chronic intoxication with valproic acid on the lipid profile is summarized in table 6. The result indicates statistically significant increase in TG ($P < 0.001$), LDL ($P < 0.001$) and VLDL ($P < 0.01$), statistically significant decrease in HDL and non-significant increase in TC in the diseases control group compared to the negative control group. Intoxication of experimental rats with valproic acid induced lipid profile alteration revealed by significant elevation ($P < 0.05$ - 0.001) in the levels of serum TG (78%), LDL

(130%), VLDL (58%) and non-significant increase TC (6%), and significant decrease in HDL (67%), compared to the negative control group. Nevertheless, sub-chronic post-treatment for with 4 weeks with NLL extract protected the rats against valproic acid induced "bad cholesterol" build up as evidenced by the reduction of lipid profile biomarkers and increase in HDL. Post-treatment with NLL 100 mg/kg body weight reduces TG (59%), LDL (58%), VLDL (67%), but increases HDL (57%) and TC (67%) significantly and non-significantly respectively. Similarly, post-treatment of NLL 200 mg/kg reduces TG, LDL and VLDL, by 68%, 75%, 81% and increases TC and HDL by 87% and 54% respectively; while post-treatment with NLL 400 mg/kg reduces these indicators (TG, LDL and VLDL) by 89%, 79%, 96% respectively but increases TC and HDL by 80% and 65% respectively. Post-treatment with Vinpocetine 25 mg/kg led to a reduction of TG, LDL and VLDL by 85%, 94%, 100% respectively and increases TC and HDL by 93% non-significantly and 54% significantly respectively in the reference control group compared to the disease control group.

| Parameters | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 |
|---|---|---|---|---|---|---|
| TC | 2.38 ± 0.13 | 2.53 ± 0.22 (6%) | 2.43 ± 0.39 (67%) | 2.40 ± 0.43 (87%) | 2.41 ± 0.38 (80 %) | 2.39 ± 0.15 (93%) |
| TG | 0.69 ± 0.07 | 1.23 ± 0.15$^{###}$ (78%) | 0.91 ± 0.05*** (59%) | 0.86 ± 0.05** (68%) | 0.75 ± 0.07*** (89%) | 0.77 ± 0.05*** (85%) |
| HDL | 1.13 ± 0.14 | 0.37 ± 0.07$^{###}$ (67%) | 0.80 ± 0.80* (57%) | 0.78 ± 0.21* (54%) | 0.86 ± 0.03** (65%) | 0.78 ± 0.14* (54%) |
| LDL | 0.40 ± 0.09 | 1.02 ± 0.21$^{###}$ (130%) | 0.82 ± 0.11** (58%) | 0.23 ± 0.08*** (75%) | 0.61 ± 0.14*** (79%) | 0.43 ± 0.05*** (94%) |
| VLDL | 0.36 ± 0.06 | 0.57 ± 0.03$^{###}$ (58%) | 0.43 ± 0.03* (67%) | 0.40 ± 0.05 (81%) | 0.37 ± 0.06*** (96%) | 0.36 ± 0.05*** (100%) |

***Table 6**: Effects of valproic acid intoxication and post-treatment with NLL hydro-methanolic extract on lipid profile of experimental albino rats following sub-chronic dosing for four weeks.*

*Group 1: Negative control group 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 NLL extract (100 mg/kg b.wu.) +valproic acid 500 mg/kg; Group 4 receiving NLL extract (200 mg/kg b.w.) + valproic acid 500 mg/kg; Group 5 receiving NLL extract (400 mg/kg b.w.) + valproic acid 500 ml/kg, Group 6: Reference control receiving vinpocetine (25 mg/kg b.w.) + valproic acid 500 mg/kg. NLL: Nauclea latifolia Leaf; TC: Total Cholesterol; TG: Triglyceride; HDL: High Density Lipoprotein; LDL: Low Density Lipoproteins; VLDL: Total Cholesterol. Values presented as mean ± standard deviation (n = 3 - 5); $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***(###)}P < 0.001$. Analysis was done using one way ANOVA and Turkey Test.*

### Effect of electrolytes and kidney function

The effects of NLL following sub-chronic intoxication with valproic acid on electrolyte levels and kidney functions are summarized in table 7. The result indicates statistically significant increase in Na ($P < 0.01$), non-statistically significant increase in K, CL, UA and CR and non-significant decrease in $HCO_3$ in the diseases control group when compared to the negative control group. Intoxication of experimental rats with valproic acid induced nephrotoxicity or nephro-cellular damage revealed by non-significant elevation in the levels of CR (15%) and non-significant decrease in UA (14%). It also affected electrolyte levels non-significantly by increasing $Na^+$, $K^+$ and $HCO^{3-}$ by 10, 18 and 2% respectively when compared to the negative control group. Nevertheless, sub-chronic post-treatment for 4 weeks with NLL extract protected the rats against valproic acid induced-nephrotoxicity as evidenced by the reduction in CR and increase in UA. Post-treatment with NLL 100 mg/kg body weight reduces $Na^+$ (93%) and $K^+$ (45%) but increases $Cl^-$ (42%), $HCO_3^-$ (375%), CR (76%) and UA (17%). Similarly, post-treatment of NLL 200 mg/kg reduces $Na^+$ (76%) and $K^+$ (40%) but increases $Cl^-$ (116%), CR (86%), UA (17%) and has no effect on $HCO_3^-$ while post-treatment of NLL 400 mg/kg reduces $Na^+$ (191%), $K^+$ (58%), $Cl^-$ (95%) and CR (80%) but increases UA (47%) and has no effect on $HCO_3^-$. Vinpocetine reduces $Na^+$ (74%) and $K^+$ (109%) but increases $Cl^-$ (200%), CR (90%), $HCO_3^-$ (1250%), UA (333%) in the reference control group respectively compared to the disease control group.

| Parameters | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 |
|---|---|---|---|---|---|---|
| $Na^+$ | 115.6 ± 6.4 | 127.5 ± 1.3## (10%) | 116.5 ± 1.3* (92%) | 118.5 ± 5.7 (76%) | 104.8 ± 2.3*** (191%) | 118.7 ± 1.2 (74%) |
| $K^+$ | 7.5 ± 1.2 | 8.9 ± 0.8 (18%) | 8.28 ± 0.7 (45%) | 8.35 ± 0.8 (40%) | 8.10 ± 1.3 (58%) | 7.4 ± 1.4 (109%) |
| $Cl^{-1}$ | 33.4 ± 2.3 | 31.5 ± 1.0 (6%) | 32.3 ± 4.0 (42%) | 33.7 ± 2.6 (116%) | 33.3 ± 1.0 (95%) | 35.3 ± 1.0 (200%) |
| $HCO_3^{-1}$ | 26.4 ± 2.6 | 26.0 ± 1.6 (2%) | 27.5 ± 4.4 (375%) | 26.0 ± 2.8 (175%) | 26.0 ± 3.7 (225%) | 31.0 ± 3.6 (1250%) |
| UA | 4.4 ± 0.7 | 3.75 ± 1.4 (14%) | 3.9 ± 0.9 (17%) | 4.0 ± 0.4 (17%) | 4.2 ± 0.8 (67%) | 5.8 ± 2.7 (333.30%) |
| CR | 164.2 ± 11.2 | 140.0 ± 10.2 (15%) | 158.5 ± 11.5* (76%) | 160.8 ± 7.7 (86%) | 159.3 ± 13.5* (80%) | 161.7 ± 21.6 (90%) |

***Table 7**: Effects of valproic acid intoxication and post-treatment with NLL hydro-methalonic extract on kidney functions and electrolyte levels of experimental rats following sub-chronic dosing for four weeks.*

*Group 1: Negative control group 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 NLL extract (100 mg/kg b.w.) +valproic acid 500 mg/kg; Group 4 receiving NLL extract (200 mg/kg b.w.) + valproic acid 500 mg/kg; Group 5 receiving NLL extract (400 mg/kg b.w.) + valproic acid 500 ml/kg, Group 6: Reference control receiving vinpocetine (25 mg/kg b.w.) + valproic acid 500 mg/kg. NLL: Nauclea latifolia Leaf; Na: Sodium, K: Potassium; Cl: Chlorine; $HCO_3$: Tricarboxylic Acid; UA: Uric Acid; CR: Creatinine. Values presented as mean ± standard deviation (n = 3 - 5); $^{*}P< 0.05$, $^{**(\#\#)}P < 0.01$, $^{***}P < 0.001$. Analysis was done using one way ANOVA and Turkey test.*

## Liver, kidney and lungs histopathology

Liver histopathology was evaluated by examination of the gross macroscopic and microscopic anatomical features. Slightly notable morphological lesions were observed in the liver diseases control group (500 mg $kg^{-1}$ $d^{-1}$). Infrequent dispersed liver steatosis was also observed in the periportal and pericentral zone of the liver in the diseases control group. These histological changes were further accompanied by mild infiltration with inflammatory cells (Figure 1). The kidney (Figure 2) did reveal substantial pathology as well as the lungs (Figure 3).

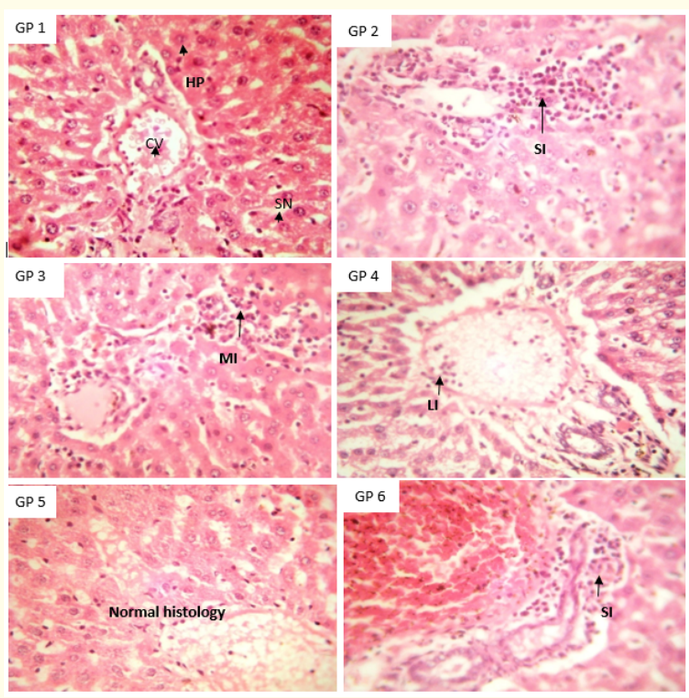


***Figure 1:** Photomicrograph of heamatoxylin and eosin stained liver tissue (x 400 magnification).*
*Group 1 shows a normal liver tissue with normal hepatocytes showing a normal nuclei cytoplasmic ratio and central vein with radiating sinusoids devoid of inflammatory cells. Group 2 shows massive influx of inflammatory cells around the central vein causing deformation of the central vein. Group 3 shows moderate inflammatory response to the injury. Group 4 shows low inflammatory response while group 5 revealed features of normal histology as seen in group 1. Group 6 shows marked inflammatory response. Valproic acid induces liver injury as seen in group 2, Group 3 and group 6 has no protection while group 4 and 5 showed hepatoprotection. Key: SI= severe inflammatory response; MI= moderate inflammatory response; LI= low inflammatory response; CV= central vein; HP = hepatocytes; SN= sinusoids; GP = group.*

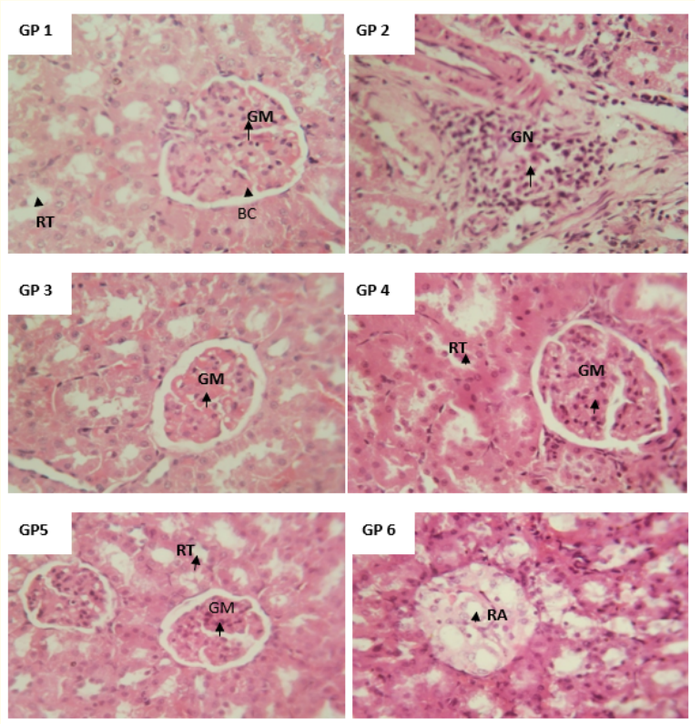


***Figure 2:*** *Photomicrograph of hematoxylin and eosin stained kidney tissues (x 400 magnification).*
*Group 1 shows normal renal tissue with abundant tubules with normal epithelium. The glomerulus shows intact Bowman's capsule consistent with normal histology of the kidney. Group 2 marked glomerulus nephritis. Group 3, 4 5 presented features consistent with normal histology. Group 6 interaction between valproic acid and standard drug resulted in atrophy. Extract is renal protective. Key: GM= glomerulus, RT= renal tubule, RA= tubular atrophy, BC= Bowman's capsule, GN= glomerulus nephritis; GP = group.*

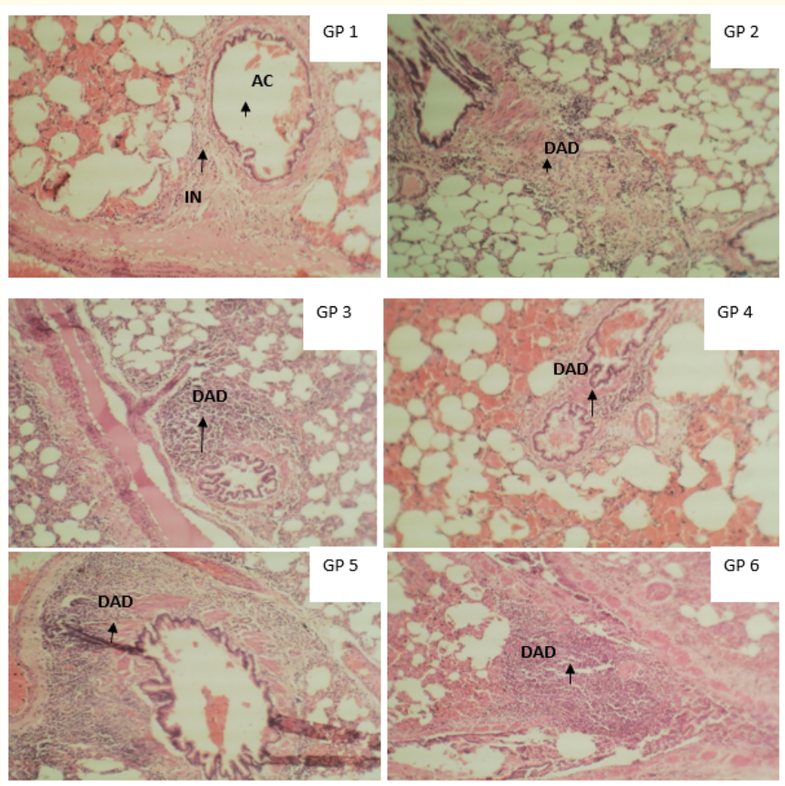


***Figure 3:*** *Photomicrograph of hematoxylin and eosin stained lung sections (x 400 magnification).*
*Group 1 shows a lung tissue showing the respiratory portion, the alveoli with a normal interstitial tissue, alveolar sac and epithelium consistent with normal histology. Group 2 shows diffuse alveolar damage with numerous inflammatory cells. Group 3,4 ,5 and 6 exhibited the same pathology seen in group 2. Extract and standard drugs are not protective. Key: DAD= diffuse alveolar damage, AC= alveolus sac, IN= Interstitial; GP = group.*

## Discussion

One of the histone deacetylase inhibitors commonly prescribed medication for management of epilepsy, migraine and bipolar disorder is Valproic acid (VPA). 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). VPA-induced pancreatitis does not depend on valproic acid serum level and may occur any time after onset of therapy. We proposed that NLL antioxidant exhibit potentials for abrogating or mitigating the global toxicity effects of VPA. Against this background NLL was examined in VPA-induced intoxication during sub-chronic oral dosing for 4 weeks in rats. The investigation indicated general increase in animal weights of all the groups but only 200 mg/kg group and 400 mg/kg showed significant increase in weight on the 1$^{st}$ week ($P < 0.05$). They also showed significant weight increase on the 4$^{th}$ week ($P < 0.05$) while group 6 showed significant weight increase in weeks 3 and 4 ($P < 0.01$ - 0.001). Drug-induced body weight changes posit as an important indicator of drug effects. Studies observing changes in body weights in intoxicated experimental animals as an indication of signs of drug toxicity abounds in literature [49-51]. This investigation is consistent with changes in weight observed in valproate acid-induced toxicity in rodents [17]. Changes in weight might be hinged on bioavailability of nutrients and modulation of appetite either by promoting or discouraging food intake. However relative organs weights were not affected. Neither VPA-induced intoxication nor posttreatment with NLL did demonstrate any significant alterations in hematological indices. It might have an impingement but probably there was high degree of recovery form toxicological insults.

Moreover, for the liver enzymes, there was marked elevation in the levels of GGT, AST, ALT, ALP and TP in the diseases control group following sub-chronic intoxication with VPA in rats. Significant elevations of liver enzymes are well known marked indicators of hepatotoxicity or liver injury. Reports indicated upregulations of liver enzymes with putative hepatotoxicants [52,53]. The insults on the histology of the hepatocytes lead to alteration of the cytoarchitecture of the cell membrane provoking cellular permeability and leakage and eventual liver damage. The damage of hepatocytes leaked cytoplasmic contents into the plasma, liver enzymes, therefore causing the elevation of these enzymes in the plasma. However, on administration of the NLL extract, there was a marked depression in the levels of these enzymes in the plasma for all the doses of the extract, comparable to that obtained with the control drug (vinpocetine).

On lipid profile, Valproic acid (VPA) have been investigated for potential hepatotoxicity in rats. VPA was reported to be potent inducers of microvesicular steatosis in young rats [54]. The drug did, however, induce hepatic lipid accumulation in mature rats. β-oxidation inhibition and several other biochemical alterations were observed in rats dosed with VPA. It was suggested that β-oxidation inhibition observed in both VPA-treated rats occurred by different mechanisms. VPA inhibits by a transient sequestering of CoA and inhibit specific enzyme(s) in the β-oxidation sequences [54]. The sub-chronic intoxication of rats with VPA demonstrated marked decreased in the level of HDL and an increase in the levels of LDL, VLDL and TG in the disease control group when compared to the negative control group. The VPA mediated inhibition of β-oxidation of free fatty acids (FFAs) might in part result in increased turnover of LDL, VLDL and TG. For TG it corroborates other report of Zhang., *et al.* [55] but our study corroborated VPA mediated depression of TC as observed in the diseases control compared to the normal control group which was upregulated non-significantly by NLL extract. The effects of VPA on lipid biomarkers apart TC were significantly ($P < 0.05$ - 0.001) reversed on administration of the NLL extract positing in part a plausible mechanism of NLL antagonizing the sequestrating effect of VPA on hepatic mitochondrial beta-oxidation of CoA and or countering its action on specific enzymes in the β-oxidation sequence [55]. This study confirms the report of VPA-mediated hepatotoxicity by diminution of TC, elevation of TG due to compromised in FFA transport machinery, provoking accumulation of FFAs aggravating liver insults and hepatic steatosis [55].

The sub-chronic intoxication of rats with VPA elicited marked elevation of $Na^+$, $K^+$, and $Cl^-$ but slightly downregulated the level of $HCO_3^-$ in the experimental group compared to the normal control group. However, the post-treatment with NLL extract downregulated significantly the levels of $Na^+$, $K^+$ and $Cl^-$ and depressed the level $Na^+$ levels in the disease control groups when compared to the negative control group. Studies have revealed that $Cl^-$ and $K^+$ fluxes play a crucial role in synaptic inhibition, cell pH regulation, as well as in cell volume control and tissue susceptibility to seizures [56,57]. However, a recent study demonstrated that VPA promote diuretic effect due to enhancement of sodium and $Cl^-$ excretion with urine [58,59]. Acute and subacute administration of valproic acid is reported to exert a moderate diuretic effect on rats [59-61]. This study demonstrates that NLL extract counteracts the diuretic effects of VPA as it downregulate the elevated $Na^+$, $K^+$, and $Cl^-$ which mediate VPA-induced diuresis.

The effect of VPA on kidney function indices showed both a decrease in the level of urea and increase in the level of creatinine but fail to reach statistical significance. Urea is primarily synthesized in the liver mitochondria and the levels depend on the concentration of ATP, which serve as biomarkers of integrity of mitochondria function. The level of urea in the urine has been observed to decrease in VPA-intoxicated rat [55]. This might in part originate from the VPA-induced downregulation of urea in the plasma observed in our study. However, the blood plasma creatinine (CA) in the VPA-induced hepatotoxicity indicate marked depression in the diseases control group compared with the normal control group. This is consistent with the study of Speir., *et al* [62]. NLL treatment in VPA-intoxicated rats ameliorated this effect across the various doses utilized, but only the 100 mg $kg^{-1}$ $d^{-1}$ and 400 mg $kg^{-1}$ $d^{-1}$ were significant ($P < 0.05$). Though, the creatinine level is influenced by both renal and non-renal factors and it is not reflective of glomerular filtration rate (GFR); it is not an optimal marker of kidney injury [63].

At the histological level the extract did appears to be effective in reversing the VPA induced insults in the liver, the kidney but not the lungs. Probably, there was an interaction between VPA and referenced drug vinpocetine, as it was not effective in ameliorating alterations on the histology of tissues investigated ( liver, kidney and lungs).

In the liver, tissue with normal hepatocytes with normal nuclei cytoplasmic ratio and a central vein with radiating sinusoids devoid of inflammatory cells was observed. The hepatotoxic group 2 revealed massive influx of inflammatory cells around the central vein causing deformation of the central vein (hepatotoxic) ; group 3, 4, 5 shows moderate, low and mild inflammatory response to the injury. Group 6 shows marked inflammatory response and vinpocetine was not heptoprotective. Valproic acid induces liver injury that was not protected by NLL extract of group 3 (100 mg/kg) while group 4 (200 mg/kg) and 5 (400 mg/kg) showed hepatoprotection but group 6 has no protection

In the kidney sections abundant glomerulus and tubules with normal epithelium and glomerulus showing intact Bowman's capsule consistent with normal histology of the kidney but the diseases control group 2 revealed marked glomerular nephritis but post-treatment with NLL extract in groups 3, 4 5 presented features consistent with normal histology but group 6, vinpocetine, the standard drug interacted with valproic acid and resulted in cellular atrophy; NLL extract at all doses were renoprotective.

In the lungs VPA- induced congestion with marked increase of goblet cells and moreover the basement membrane was thickened with heavy accumulation of inflammatory cells. The extracts and the reference drugs did not posit any observable protective effect. Taking together in VPA induced toxicity at the lungs the NLL extract at the concentration used did not appears to possess capacity to initiate any alteration of pathological insults or impingement contrary to demonstrable effectiveness observed on the biochemical profile of the liver and the kidney.

Taking together on the probable mechanistic modulation of the observed effect on the liver and kidney but not the lungs; is valproic acid (VPA) a histone deacetylase inhibitor inhibiting sirtuin 1 (histone deacetylase) that is essential for liver, kidney and lung functions? Sirtuin 1 is critical to maintenance of glucose homeostasis as SIRT1 is required for induction and maintenance of fatty acid oxidation in response to low glucose concentrations [64]. Valproic acid has antidiabetic properties relevant to glucose homeostasis as HDAC inhibitor is reported to provoked suppression of insulin secretion at low glucose concentrations and enhance insulin secretion at high glucose concentrations. Thus, HDAC inhibitor when used clinically, at high doses is reported to provoke hypoglycemia in the fasting state and hyperglycemia in the fed state. Therefore, physicians should be aware of the capacity of these drugs to modulate the insulin secretory capacity of pancreatic beta cells [65]. However, higher doses of VPA may lead to Sirtuin 1 inhibition. Is it likely that NLL extract could activate Sirtuin 1 to reverse the VPA HDAC inhibition and maintain the function of the liver, kidney and lung? This is possible because sirtuin 1, is a deacetylase, and is involved in several metabolic and signaling proteins involved in stress response, apoptosis, mitochondrial function, self-renewal, and neuroprotection [66]. The NLL extract role might perhaps be acting to counteract the effect of VPA as HDAC inhibitor to improve the function of the liver and kidney but not the lungs as observed when VPA was supplemented along with NLL extract. The probable mechanism of NLL extract might in part be upregulating the expression or turnover or increasing the synthesis of sirtuin 1 or enhancing the deacetylation effect culminating in the activation of Sirtuin 1.

The other question could be whether NLL extract could be associated with maintenance of glucose homeostasis with relevant Sirtuin 1 activation and pancreas function? Emphatically yes, the effect of NLL extract on glucose homeostasis has been reported [30,67]. This

study indicate that NLL extract abrogates potential VPA - induced inhibition on the histology of liver underpinning NLL extract potential mechanism of action as an activator of Sirtuin 1, a similar mechanism of action exhibited by metformin, an oral hypoglycemic agent [68]. Besides sirtuin 1 is reported to be downregulated in cells that have high insulin resistance [69,70]. Therefore, supplementation of NLL extract could modulate Sirtuin 1 corroborating reports of its anti-hyperglycaemic effects and probable modulation of pancreatic function in diabetes mellitus [71-73].

The presence of alkaloids, saponin, terpenoid, flavonoid, steroid, and cardiac glycosides as revealed in the hydro-methanolic NLL extract might in part be working in synergy to ameliorate VPA-induced toxicity at the biochemical levels and probably also at the histological level of the various organs investigated. In addition to resveratrol, a range of other plant-derived polyphenols has also been shown to interact with SIRT1 [64]. This investigation corroborates the antidiabetic effect of the NLL extract in experimental animals [67,74].

## Conclusion

The NLL extract demonstrated to larger extent the potentials for alleviating multifocal alterations induced by VPA on the animal weights, biochemical parameters, and the histological cyto-architecture of the liver and kidney but not the lungs. The supplementation of NLL extract along with VPA attenuated VPA-induced pan-toxicity in the diseases control group, positing probable activator role on sirtuin1, as NLL extract might in part be counteracting the effect of VPA on sirtuin 1. The activation of sirtuin 1 might be responsible for the protective effects on the liver, kidney but not the lung functions. The plausible upregulation of sirtuin 1 corroborate previous antidiabetic report of NLL extract and its conceivable critical role in the maintenance of glucose homeostasis. Thus, NLL extract has capacity for hepatoprotection and renoprotection but not pulmonary protection across different systemic milieu prone to VPA -induced toxicity. Further study to histochemically characterize NLL effect on sirtuin 1 protein expression in VPA induced -toxicity is underway in our laboratory.

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**Volume 7 Issue 5 May 2019**