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Phenolics-rich extracts of *Nauclea latifolia* fruit ameliorates lead acetate-induced haematology and lung tissues toxicity in male Wistar rats

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ABSTRACT

Lead toxicity is a worldwide problem that needs to be ameliorated with harmless or less toxic medications contrary to available drugs. As such, ameliorated effects of methanol and methanol/acetone phenolic-rich extracts of *N. latifolia* fruit (NLF) was assessed on haematological parameters and lung histology of lead acetate induced toxicity rats after acute toxicological study and phenolics analysis of the extracts. Seven groups of Wistar rats comprising of 5 rats each were used for the study. Dosages of 150 mg/kg and 300 mg/kg body weight (bw.) of both extracts NLF were administered orally to four of the groups while two groups were administered 100 mg/kg bw. of silymarin and 2 mL/kg bw. of normal saline (Untreated) followed with control group for 14 days. Phenolics contents (mg/g) of both extracts showed the following order; tannins < flavonoids < total phenols (9.29 < 10.02 < 23.82) and (10.72 < 18.09 < 61.39) for both methanol and methanol/acetone phenolic-rich extracts of NLF respectively with LD_{50} > 2000 mg/kg bw. Significant depression of PCV, RBC, MCHC, HB, MCV, MCH, platelet counts, neutrophils and lymphocytes counts with elevation of WBC were observed in untreated group when compared with non-toxic control group. The administration of both extracts of NLF at 150 and 300 mg/kg bw. were capable of reverting the haematological changes in lead acetate induced toxicity rats with minimal injury to the lung tissues. Therefore, methanol and methanol/acetone phenolic-rich extracts of NLF can restore haematological abnormalities as well as sustain the integrity of the lung tissues of the experimental rats. Hence, phenolics content of *N. latifolia* fruit can be exploited further for drug development for the management of lead toxicity.

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Introduction

Lead is a heavy metal with known toxicological effect which is widely recognized as an industrial toxicant which is well-known and widely spread all over the universe [1]. There is increase in lead circulation in water, air and soil due to human events associated with smoking, food, manufacturing, drinking water, and other domestic sources [2]. Lead has been found to produce a wide range of adverse biochemical effects involving several organs, systems and biochemical activities in all mammals [3].

The alteration of the haem synthesis is an early effect linked with elevation of lead concentration in the soft tissues or organs. Inhibition of δ-aminolaevulinic acid dehydratase (ALAD) as well as elevation of protoporphyrin in erythrocytes are the initial effects, followed by increased δ- aminolaevulinic acid (ALA) and urinary coproporphyrin excretion [4].

Polyphenol compounds from herbal vegetations are one of the main sources of antioxidant which may assist in protection of the cells from oxidative impairment triggered by free-radicals [5]. The metabolites are known to be reducing agents, metal chelators, singlet oxygen quenchers, radical scavengers and hydrogen donors [6]. In order to extract maximum amount of this secondary metabolites from various plants, several solvents of differing polarities must be used [7]. Several scientists found out that methanol (CH_3OH) was highly active for large amount extraction of phenolic contents from fruits of some plants in comparison with ethanol [8,9]. However, extraction of Ivorian plants with ethanol extracts give higher concentrations of phenolics when compared with water, acetone and methanol [10]. Furthermore, the mixture of more solvents could as well stimulate the yields of phenolics as proved in increase yield of phenolics from wheat bran with 80 % ethanol and water [11,12]. In another case, 50 % of water-ethanol was able to extract more phenolics from aerial parts of *P. atrosanguinea* than 50 % aqueous methanol, and acetone or pure form of each solvent [13]. However, absolute methanol and 80 % water-acetone were able to extract highest concentration of phenolics from sunflower meal and *V. vinifera* wastes respectively [14,15]. These changes in concentration of extracted phenolics might be as a result of the properties of the phenolic contents of the plants in question as related to various solvents used.

Nauclea latifolia (African peach) is of the family Rubiaceae; a small tree or straggling shrub from tropical Asia and Africa. African peach fruit contains vitamins E, A, B2, C and B1 and other valuable secondary metabolites [16]. Several parts of *N. latifolia* have been reported to have the following activities; antimicrobial [17] antiulcer [16], and antihypertensive [18,19]. The sticks are used as chewing stick and a remedy against tuberculosis [20], hypocholesterolaemia [21], diabetes [22] and hepatic damage [23,24].

Therefore, the aim of this research is to assess the ameliorative properties of phenolics in *N. latifolia* fruit on haematological parameters and histology of the lung of lead acetate-induced toxicity rats.

Materials and methods

Materials

Plant collection

Fruits of fresh matured *Nauclea latifolia* were collected from Bosso Estate in Bosso Local Government area of Niger State (Latitude: 9.6550611 and Longitude:6.5138343) *Nauclea latifolia* fruit was identified by a Senior Botanist of the Biological Science Department of Federal University of Technology Minna with voucher number: FUT.MIN/SLS/PB-019-016 and the specimen was deposited at the herbarium of the University.

Chemicals and reagents

The chemicals and reagents used were of analytical grade which were obtained from Sigma-Aldrich Chemical Company St. Louis, USA. The reagents include methanol, acetone, lead acetate, ethanol, quercetin, tannic acid, gallic acid, sodium carbonate, aluminum chloride, sodium hydroxide, hematoxylin and eosin.

Experimental animals

Male Wistar rats (125.00 $\pm$ 3.16) g were obtained from the animal facility of Federal University of Technology Minna, Niger State, Nigeria. The animals were housed in polypropylene cages under a controlled environment with 12 h light/dark cycles, temperature of 28 $\pm$ 3 $^{\circ}$C and relative humidity of 45-50 %. The animals were fed on pelleted diet (Vital Feeds, Jos Nigeria) with supply of water *ad libitum*. The experiment was conducted according to protocol review (1997) of Canadian Council on Animal Care and use guidelines as described by Kabiru *et al.* [25]. Ethical clearance number **0000012EAU** was given by Federal University of Technology, Minna/Nigeria Ethical Review Committee in accordance with international standard on the care and use of experimental animals.

Methods

Sample preparation

Fruits of matured *N. latifolia* fruits were washed, sliced into pieces and dried under room temperature. The dried fruits were milled using electric blender (Binatone BLG 450, United Kingdom). The powdered sample was kept in an air tight plastic container at room temperature (28 ± 2)°C until further use

Extraction of phenolics from N. latifolia *fruits*

Fifty grams (50 g) of the powdered sample was placed in a round bottom flask and macerated with 400 mL of 70 % methanol and another 50 g of the same sample was macerated with 40 % acetone and 60 % methanol mixture at room temperature for 72 h with occasional agitation. The extracts were filtered with muslin cloth after which the methanol or methanol/acetone solvent were evaporated at low pressure in a rotary vacuum evaporator. The filtrate of *N. latifolia* fruit obtained were lyophilized to obtain phenolics rich extract which was stored at -4°C.

Quantification of Phenolic Compounds

Quantification of ***total phenol***

Total phenol contents of both extracts were quantified according to McDonald et al. [26]. Briefly, exact 0.5 mg/mL of either extracts was dissolved in CH_3OH and each of the dissolved sample was mixed with exact 2.9 mL of 2 %Na_2CO_3. The mixture was incubated in a laminar flow at room temperature for two minutes followed by addition of 0.1 mL of 0.2 N Folin-Ciocalteau reagent. The mixture was then incubated in a laminar flow for another 30 min at 37 °C. The absorbance of the reaction mixture was taken at 750 nm with a Shimadzu UV-Spectrophotometer UV-1800 model (Japan). Exact 0.5 mg/mL of gallic acid in methanol was used as standard. The phenolics content of both extracts were expressed as milligram gallic acid equivalents (mg gallic acid/g extract). All samples were analysed in triplicates.

Quantification ***of flavonoids***

The flavonoids content was quantified as described by Ferreira, et al. [27] using spectrophotometric techniques. About 0.5 mg/mL of the extract and quercetin were prepared in absolute methanol and 250 μL of each of mixture was mixed with deionized water (1.6 mL) and 100 μL of 5 %NA_2CO_3**.** The samples were left for 6 min after which 150 μL of 10 % $AlCl_3$ solution was pipetted in to the mixture followed by 5 min incubation at 37 °C. About 500 μL of 1 M NaOH was added to halt the reaction followed by 15 min incubation at 37 °C. The absorbance of the resulted mixture was read at 510 nm using a Shimadzu UV-Spectrophotometer against the blank (contained 250 μL of absolute methanol instead of the extract or quercetin). The 0.5 mg/mL of quercetin in methanol was used as standard. The flavonoids content was quantified as milligram quercetin equivalents (mg quercetin/g extract).

Quantification ***of*** *tannins*

The procedure used in determining the tannins contents was adopted from Sofowora [28]. A 0.2 g of each extract was weighed into a 50 mL beaker followed by addition of 20 mL of 50 % methanol. The beakers were covered with aluminum foil and placed in a water bath with shaker at 80 ºC for 1 hour. The extract was allowed to cool, filtered with double layered whatman No.41 filter paper into a 100 mL volumetric flask with addition of 20 mL of water followed by the addition of Folin-Denis reagent (2.5 mL) and 17 %NA_2CO_3(10mL). Water was added to the mixture to100 mL volume and allowed to stand for 20 mi for colour development after proper mixing; the absorbance was read against blank with spectrophotometer at 760 nm. The same procedure was followed for tannic acid standard.

Acute toxicological study of the NLF

Acute toxicity test was performed using Organisation for Economic Co-operation and Development (OECD) protocol. The behavioural profiles (alertness, restlessness, irritability and fearfulness), neurological (spontaneous activities, reactivity, touch response and pain response) and autonomic profile (defecation and urination) and mortality were monitored during the experiment [29]

Animal Grouping for lead acetate-induced haematology and lung tissues toxicity

The Wister rats were grouped into seven of five rats each as shown below;

Group 1 (Control): 0.5 mL/kg bw. of Normal saline
Group 2 (Silymarin): Lead acetate (1000 mg/kg bw.) followed by 100 mg/kg bw. Silymarin

Table 1
Phenolics Contents of methanol and methanol/acetone phenolic-rich extracts of *N. latifolia* fruit (NLF).

Phytochemicals	Concentration (mg/g)	
	Methanol extract	Methanol/Acetone Extract
Tannins	9.29±0.39	10.72±0.21
Total phenols	23.82±1.21	61.39±0.28
Flavonoids	10.02±0.19	18.09±0.03

Values are expressed as mean ± standard error of mean (SEM) of five replicates

Group 3 (Untreated): Lead acetate (1000 mg/kg bw.) followed by 0.5 mL/kg bw. of Normal Saline
Group 4 (150 mg/kg bw. Methanol Extract of NLF): Lead acetate (1000 mg/kg bw.) followed by 150 mg/kg bw. Methanol extract of phenolics-rich *N. Latifolia* fruit
Group 5 (300 mg/kg bw. Methanol Extract of NLF): Lead acetate (1000 mg/kg bw.) followed by 300 mg/kg bw. Methanol extract of phenolics-rich *N. Latifolia* fruit
Group 6 (150 mg/kg bw. Meth./Acet. Extract of NLF): Lead acetate (1000 mg/kg bw.) followed by 150 mg/kg bw. Methanol/Acetone extract of phenolics-rich *N. Latifolia* fruit
Group 7 (300 mg/kg bw. Meth./Acet. Extract of NLF): Lead acetate (1000 mg/kg bw.) followed by 300 mg/kg bw. Methanol extract of phenolics-rich *N. Latifolia* fruit.

The 1000 mg/kg bw. of lead acetate was orally administered to induce lead toxicity in six groups for 14 days. Each extract of 150 mg/kg bw. and 300 mg/kg bw. were administered to four of the groups after one hour of lead acetate administration. One of the remaining two groups of the lead induced toxic group was treated with 100 mg/kg bw. of silymarin (Standard drug) while the other one (Untreated) was given 2 mL/kg bw. of 0.9 % saline solution. The animals were euthanized by cardiac puncture at 15th day after an overnight fast while blood and lung were harvested for haematological and histological analysis respectively.

Histology of the lung

A section of lung tissues was quickly fixed in 10% neutral buffered formalin solution prior to at least 24 hours of examination. The fixed specimen was processed by using conventional paraffin embedding technique (dehydration via ascending grades of C_2H_5OH, clearing using chloroform and embedding with paraffin wax at 60 ºC. Paraffin blocks was prepared from which 3-4 μm thick sections was gotten and stained with haematoxylin and eosin (H&E). The histology observations were then made under light microscope at Magnification x 40.

Data analysis

The analysis of variance (ANOVA) followed by *Post hoc* Duncan multiple comparisons test (Statistical Package for Social Sciences, version 22.0, SPSS Inc., Chicago, IL, USA) at 95% confidence interval was used on the data obtained with *p*-value less than 0.05 was considered significant difference. The data were expressed as mean ± standard error mean of five replicates.

Results

The phenolics contents of methanol and methanol/acetone phenolics-rich extracts of *N. latifolia* fruit (NLF) is shown in Table 1. Total phenols, flavonoids and tannins are present in both samples in descending order (total phenol > flavonoids > tannins). However, the concentration (mg/g) of these phenolics are actually more in the methanol/acetone extract (61.39 > 18.09 > 10.72) when compared with methanol extract (23.82 > 10.02 > 9.29) of NLF.

Acute toxicity test

The result of acute toxicity test showed normal behavioural profiles (alertness, restlessness, irritability and fearfulness), neurological (spontaneous activities, reactivity, touch response and pain response) and autonomic profile (defecation and urination) are all normal during the side cage observation and beyond. Although, they were less active within the first four hours but their activities became normal afterwards. No mortality was recorded throughout the fourteen days of observation at 2000 mg/kg bw. of both extracts.

The Haemoglobin Concentration (HB), Packed Cell Volume (PCV), Red Blood Cell Counts (RBC) of male Wistar rats administered with methanol and methanol/acetone phenolic-rich extracts of *N. latifolia* fruit (NLF)

The Haemoglobin Concentration (HB), Packed Cell Volume (PCV), Red Blood Cell Counts (RBC) of male Wistar rats exposed to lead acetate and treated with methanol and methanol/acetone (Meth./Acet.) phenolic-rich extracts of *N. latifolia* fruit (NLF) are shown in Table 2. Administration of lead acetate causes significant reduction ($p < 0.05$) in HB (7.02 g/dL),

Table 2
Haemoglobin Concentration (HB), Packed Cell Volume (PCV), Red Blood Cell Counts (RBC) of male Wistar rats administered with lead acetate and treated with methanol and methanol/acetone phenolic-rich extracts of *N. latifolia* fruit.

Treatments	HB (g dL^{-1})	PCV (%)	RBC (x10^6 μL^{-1})
Control (2 mL/kg bw. Normal Saline)	13.83±0.07^{b}	44.33±0.67^{e}	6.93±0.07^{bc}
Silymarin (100 mg/kg bw.)	11.03±0.23^{b}	34.33±0.33^{b}	6.53±0.13^{c}
Untreated (2 mL/kg bw. Normal Saline)	7.02±2.51^{a}	28.00±0.58^{a}	4.70±0.64^{a}
150 mg/kg bw. Methanol Extract of NLF	10.70±0.75^{b}	33.00±1.73^{b}	6.55±0.49^{b}
300 mg/kg bw. Methanol Extract of NLF	11.47±0.26^{b}	36.50±0.29^{d}	7.20±0.58^{bc}
150 mg/kg bw. Meth./Acet. Extract of NLF	10.77±0.33^{b}	35.00±0.00^{c}	6.77±0.03^{bc}
300 mg/kg bw. Meth./Acet. Extract of NLF	12.20±0.17^{b}	39.00±0.58^{d}	7.50±0.35^{bc}

Values are Means of five replicates ± SEM

Table 3
Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) of male Wistar rats administered with lead acetate and treated with methanol and methanol/acetone phenolic-rich extracts of *N. latifolia* fruit.

Treatments	MCV (fl)	MCH (pg)	MCHC (g/dL)
Control (2 mL/kg bw. Normal Saline)	68.00±2.00^{f}	27.00±0.00^{d}	38.67±0.33^{e}
Silymarin (100 mg/kg bw.)	62.67±3.33^{e}	23.00±2.00^{c}	34.00±1.00^{cd}
Untreated (2 mL/kg bw. Normal Saline)	43.50±0.29^{a}	16.00±0.30^{a}	23.50±2.60^{a}
150mg/kg bw. Methanol Extract of NLF	54.00±0.58^{b}	21.00±1.73^{b}	29.50±0.29^{b}
300 mg/kg bw. Methanol Extract of NLF	59.50±4.33^{c}	23.50±2.60^{c}	31.50±0.29^{c}
150 mg/kg bw. Meth./Acet. Extract of NLF	58.30±0.58^{c}	19.00±0.10^{b}	30.00±0.00^{bc}
300 mg/kg bw. Meth./Acet. Extract of NLF	59.00±0.00^{c}	22.50±0.29^{bc}	34.50±0.29^{d}

Values are Means of five replicates ± SEM

PCV (28.00 %) and RBC (4.70×10^6 μL^{-1}) when compared with non-toxic control group with 13.83 g/dL, 44.33 % and 6.93×10^6 μL^{-1} respectively. However, the administration of both *N. latifolia* fruit extracts significantly restored the values of all these parameters into normal or towards normal and some of the extracts treated groups are comparable to the standard drug Silymarin.

Values with different superscripts and on the same column are significantly different (p < 0.05).

The Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) of male Wistar rats administered with methanol and methanol/acetone phenolic-rich extracts of *N. latifolia* fruit

The Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) of male Wistar rats shown in Table 3. A significant decrease in MCV, MCH and MCHC ($p < 0.05$) were observed (43.50 fl, 16.00 pg and 23.50 g/dL) when compared with non-toxic control group of 68.00 fl, 27.00 pg and 38.67 g/dL respectively. However, the aforementioned parameters significantly restored ($p < 0.05$) after treated with *N. latifolia* fruit extracts. Although, the parameters are incomparable to both silymarin and non-toxic control yet methanol/acetone (Meth./Acet.) phenolics rich extract exhibits higher effect on the mentioned parameters when compared with methanol phenolics rich extract.

Values with different superscripts and on the same column are significantly different (p < 0.05).

The Platelets Count (PLC), White Blood Cell Counts (WBC), Neutrophils (N) and Lymphocytes (L) Counts of male Wistar rats administered with lead acetate and treated with methanol and methanol/acetone phenolic-rich extracts of *N. latifolia* fruit

The Platelets Count (PLC), White Blood Cell Counts (WBC), Neutrophils (N) and Lymphocytes (L) Counts of male Wistar rats administered with lead acetate and treated with methanol and methanol/acetone phenolic-rich extracts of *N. latifolia* fruit are shown in Table 4. Administration of lead acetate causes significant reduction ($p < 0.05$) in platelet counts ($660.00\times10^3/\mu L$), neutrophils (22.50 %) and lymphocytes counts (39.00 %) with increase in white blood counts ($9.40\times10^3/\mu L$) when compared with non-toxic control group with $402.33\times10^3/\mu L$, 50.67 % and 76.00 % respectively with white blood cell counts of ($4.03\times10^3/\mu L$). However, the administration of both phenolic-rich extracts of *N. latifolia* fruit significantly restored the values of all these parameters towards the standard drug treated group with higher effects among methanol/acetone phenolic-rich extracts of *N. latifolia* fruit treated groups.

Values with different superscripts and on the same column are significantly different (p < 0.05).

Histology of the lung of male Wistar rats administered with lead acetate and treated with methanol and methanol/acetone phenolic-rich extracts of *N. latifolia* fruit

The histology of the lung of male Wistar rats that was administered with lead acetate and treated with methanol and methanol/acetone phenolic-rich extract of *N. latifolia* fruit was shown in Plate 1 and 2. Administration of lead acetate to the experimental rats causes severe infiltration of thin walled of alveolar sacs with altered bronchial tissue of the lung when compared with control group (Control) and silymarin treated group that shows normal lung tissue with thin walled alveolar

Table 4
Platelets Count (PLC), White Blood Cell Counts (WBC), Neutrophil (N) and Lymphocyte (L) Counts of male Wistar rats administered with lead acetate and treated with methanol and methanol/acetone phenolic-rich extracts of *N. latifolia* fruit.

Treatments	PLC ($x10^3/\mu L$)	WBC ($x10^3/\mu L$)	N (%)	L (%)
Control (2 mL/kg bw. Normal Saline)	402.33 ± 2.33^{a}	4.03 ± 0.27^{a}	50.67 ± 0.33^{f}	76.00 ± 2.00^{g}
Silymarin (100 mg/kg bw.)	413.67 ± 3.33^{b}	4.83 ± 0.43^{c}	43.33 ± 2.67^{e}	70.00 ± 3.00^{f}
Untreated (2 mL/kg bw. Normal Saline)	660.00 ± 8.08^{e}	9.40 ± 0.23^{g}	22.50 ± 2.02^{a}	39.00 ± 0.00^{a}
150 mg/kg bw. Methanol Extract of NLF	512.50 ± 14.09^{d}	6.70 ± 0.30^{f}	30.50 ± 0.87^{b}	49.00 ± 2.89^{b}
300 mg/kg bw. Methanol Extract of NLF	460.50 ± 13.01^{c}	5.77 ± 0.58^{e}	35.50 ± 0.29^{c}	54.83 ± 5.83^{c}
150 mg/kg bw. Meth./Ace. Extract of NLF	454.67 ± 12.41^{c}	5.27 ± 0.26^{d}	36.00 ± 1.15^{bc}	61.50 ± 3.18^{ed}
300 mg/kg bw. Meth./Ace. Extract of NLF	409.67 ± 12.30^{b}	4.15 ± 0.58^{b}	38.00 ± 0.15^{d}	60.00 ± 2.46^{d}

Values are Means of five replicates ± SEM

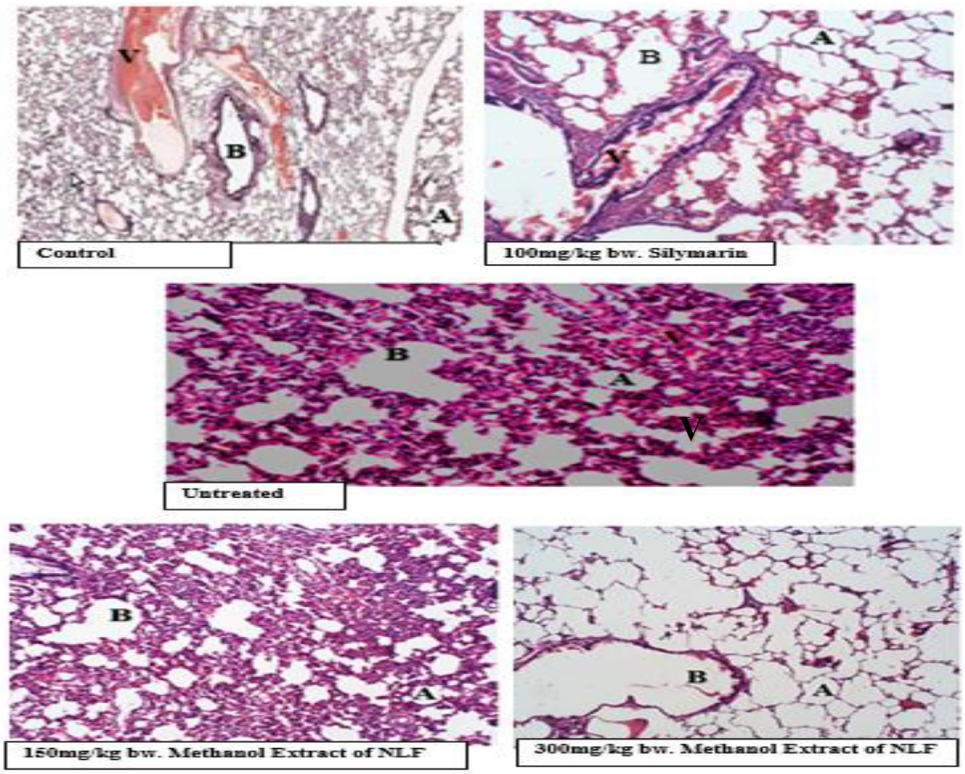

Plate 1. Histology of the lung of male Wistar rats administered with lead acetate and treated with methanol phenolic-rich extract of *N. latifolia* fruit (mg x 40) B: Bronchial tissue, A: Alveoli sac, V: Vascular space

sacs and normal bronchial tissue which composes of unaffected muscular wall and lined by pseudo stratified epithelium. However, administration of methanol phenolic-rich extracts of *N. latifolia* fruit at the dosage of 150 and 300 mg/kg bw. were actually able to prevent the lung from too much damage as revealed from the **s**ections of the lung tissue which shows moderate to severe infiltration of the alveolar walls by mixed inflammatory cells; mainly, histocytes, lymphocytes and plasmic cells.

The Administration of methanol/acetone phenolic-rich extracts of *N. latifolia* fruit at both 150 and 300 mg/kg bw. were able to cause minimal injury to the lung tissues with moderate infiltration of mostly polymorphonuclear cells.

Discussions

Heavy metals are dangerous materials that cause many health risks to the inhabitants of the ecosystems due to their high toxicity as they accumulate throughout the food chain [30]. Lead is not known to serve any necessary biological function within the body system and its presence in the body can lead to toxic effects. Lead is one of the most prevalent heavy metals contaminant with wide spread industrial and domestic uses and poisonous to almost all organs, tissue, cells (including haematopoietic cells) and can lead to death [31]. Blood or blood constituents are the best indicators of internal exposure of an individual to lead [31].

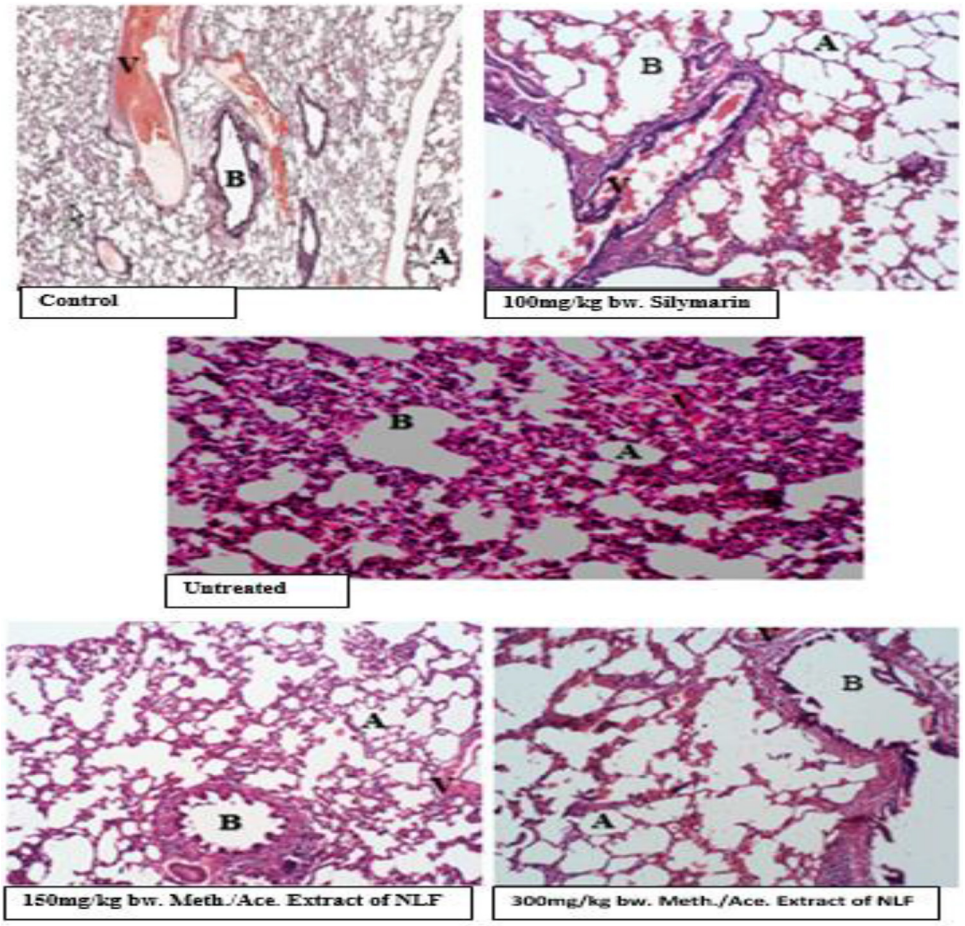

Plate 2. Histology of the Lung of male Wistar rats administered with lead acetate and treated with Methanol/Acetone Extract of *N. latifolia* fruit (Mg x 40) B: Bronchial tissue, A: Alveoli sac, V: Vascular space

The LD_{50} of both phenolics-rich NLF extracts were found to be greater than 2000 mg/kg bodyweight (LD_{50} > 2000 mg/kg.bw) with normal behavioural, autonomic and neurological profile in addition to non-mortality of the experimental animals, connotes relatively low toxicity of *N. latifolia* fruits extract. This low toxicity is actually not surprising as this really justifies the local uses of *N. latifolia* fruits extract for various ailments.

In this study, reduction in HB, PCV and RBC in untreated rat after administration of lead acetate indicate anaemic conditions [30]. The observed changes in haematological parameters can be as a result of the toxic effects of lead on; cells that involved calcium as their secondary mediator, activities of some enzymes such as aminolevulinic acid dehydratase, Glyceraldehyde 3-phosphate dehydrogenase, Glucose 6-phosphate dehydrogenase that plays a vital role in heme synthesis [31,32]. When one is continuously exposed to lead, it can adversely affect the heme biosynthesis as a result of inhibition of mitochondrial and cytoplasmic enzymes [32]. The depressing action of lead acetate on the activities of main enzymes in the biosynthesis of heme can be as a result of imperfection of metabolism of iron [30]. The lead acetate inhibits the production of protoporphyrin IX from coproporphyrinogen III and this is characterized by shortening of RBC life span and eventually affect the production of red blood cells [31]. The significant depression of haematological parameters could be as a result of binding of lead to erythrocytes, which elevate its membrane fragility that lead to its timely destruction [32]. However, treatment with methanol and methanol/acetone phenolics rich extract of NLF reversed the lead induced toxic effect by significantly increasing these stated values in the extracts treated and reference drug treated-groups. Although, the higher doses exhibited more effect compared to lower doses while methanol/acetone phenolics rich extract of NLF produced the best activities probably as a result of high concentration of phenolic compounds. The reasons behind the activities of the extracts is not mind-boggling as phenolics such as flavonoids and tannins have been reported to possess strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free-radicals [5]. Phenolics have been confirmed as metal chelators, radical scavengers, reducing agents, singlet oxygen quenchers and hydrogen donors [6]. As such, these particular secondary metabolites in the *N. latifolia* fruits might have been chelating the lead in the blood and other tissues as well as mopped up lead induced free radicals in the blood which will eventually free hematopoietic tissues

of oxidative stress. Therefore, the non-availability of lead in the tissues will actually prevent its binding to the enzymes that is responsible in haemoglobin production as manifested via improvement in haematological parameters of the NLF and reference drug treated groups. In addition, the possibilities of haematinic potentials of phenolic rich NLF could not be underestimated in the improvement of HB, PCV and RBC extract treated animals as phenolics such as tannins and flavonoids can accelerate erythropoiesis [33].

The significant depression in MCV, MCH and MCHC demonstrated followed lead acetate administration to the experimental animals is considered an early response to lead intoxication. This hypochromic microcytic anaemia was reported to be the result of depression of bone marrow activity in both animals and humans [34]. Furthermore, MCV determine the size of RBCs, while MCH indicates the concentrations as well as weight of haemoglobin in the RBCs [35]. The decrease in MCH and MCV as obtained actually reflected the previous decrease in RBC, PCV and HB observed in this study. Similarly, the decrease in MCV may be an indication that RBCs are abysmally small in size because of either the system inability to synthesize RBCs or a lack of available haemoglobin needed to complete the process of synthesizing RBCs [35]. The reversed conditions observed regarding these haematological parameters in the presence of the extract might be as a result of the aforementioned factors that were responsible for the reversion of the RBC, PCV and HB as previously explained.

Significant increase of WBC in lead acetate induced toxic rats might be as a result of the inflammatory response induced-defense mechanism in response to lead induced toxicity [36] which occurred as a result of marrow infiltration by toxic substances. The condition of elevation of leucocytes counts is called leukopoiesis which takes place in lymphoid organs as a result of increase in WBC production from the germinal center of lymphoid organs under the influence of lead toxicity and other toxicants [33]. It has been reported that lead induced inflammation could lead to increase in white blood cells count [33] as it occurred in this study. Also, the increased PLC counts could be due to erythropoietin stimulation by the increase demands of oxygen and CO_2 transport due to accelerating metabolic process or the damage of the respiratory membranes resulting to impaired gaseous exchange [37]. Likewise, significant decrease observed in neutrophils and lymphocytes of the lead acetate induced toxic rats signify serious pathological conditions such as in iron deficiency anemia [35]. The reversion of all these aforementioned parameters could be as a result of the presence of flavonoids which have been confirmed to exhibit anti-inflammatory, anti-allergic effects, antithrombotic and tumour protective principles [38].

Lung is among the target organ of lead toxicity especially through Inhalation as well as blood circulation [39]. About 90% of lead in the form of particles do enter blood circulation and get retained in the blood, however, the alveoli of the lung completely and efficiently absorb these retained lead particles [40]. The lead acetate induced alteration of histology of the lung in untreated experimental rats viz-a-viz: severe Infiltration of thin walled of alveolar sacs, altered bronchial tissues as evident from this study corroborate the retentive abilities of the lung to the toxicants. This destructive property might be due to lead induced oxidative stress in the lung resulting from free radical activities. However, the reversion of these effects after administration of methanol and methanol/acetone phenolic-rich extracts of *N. latifolia* fruit demonstrate the corrective or preventive abilities of the extract against lead induced lung damage.

The actual mechanisms at which the extracts elicit the activities are not known but the previously mentioned phenolic properties such as; antioxidant, chelating, anti-inflammatory, anti-tumour properties cannot be ruled out.

Conclusion

It can be concluded from this study that the administration of methanol and methanol/acetone phenolic-rich extracts of *N. latifolia* fruit to lead acetate induced toxic rats can restore haematological abnormalities as well as lung histology integrity of the experimental rats.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] O.U. Ademuyiwa, R.N. Ugbaja, S.O. Rotimi, E. Abam, B.S. Okediran, O.A. Dosumu, B.O. Onunkwor, Erythrocyte acetylcholinesterase activity as a surrogate indicator of lead-induced neurotoxicity in occupational lead exposure in Abeokuta, Nigeria. Environ. Toxicol. Pharmacol. 24 (2) (2007) 183–188.
[2] Nutrition Landscape Information System (NLIS) Country Profile Indicators: Interpretation Guide, World Health Organization, 2019.
[3] O. Ademuyiwa, T. Arowolo, D.A. Ojo, O.O. Odukoya, A.A. Yusuf, Lead levels in blood and urine of some residents of Abeokuta, Nigeria. Trace Elements Electrol. 19 (2) (2002) 63–69.
[4] T. Sakai, Biomarkers of lead exposure, Ind. Health. 3 (2) (2000) 127–142.
[5] R.U. Hamzah, Jigam A.A., H.A. Makun, E.C. Egwim, A.Y. Kabiru, F.M. Madaki, Secondary metabolites and In-vitro antioxidant properties of methanol extracts of fruits of *Annona senegalensis, Curcubita pepo* L, *Cucumi melo inodorous* and *Sarcocephalus latifolius*, Niger. J. Technol. Res. 13 (1) (2018) 89–96.
[6] P.Y. Wong, D.D. Kitts, Studies on the dual antioxidant and antibacterial properties of parsley (Petroselinum crispum) and cilantro (Coriandrum sativum) extracts, Food Chem. 97 (3) (2006) 505–515.
[7] Z.P. Ruan, Y.M.Lin L.L.Zhang, Evaluation of the antioxidant activity of Syzygium cumini leaves, Molecules 13 (10) (2008) 2545–2556.
[8] E. Koffi, T. Sea, Y. Dodehe, S. Soro, Effect of solvent type on extraction of polyphenols from twenty-three Ivorian plants, J. Animal Plant Sci. 5 (3) (2010) 550–558.
[9] C.P. Anokwuru, G.N. Anyasor, O. Ajibaye, O. Fakoya, P. Okebugwu, Effect of extraction solvents on phenolic, flavonoid and antioxidant activities of three nigerian medicinal plants, Nature Sci. 9 (7) (2011) 53–61.

[10] P. Garcia-Salas, A. Morales-Soto, A. Segura-Carretero, A. Fernández-Gutiérrez, Phenolic-compound-extraction systems for fruit and vegetable samples, Molecules 15 (12) (2010) 8813–8826.
[11] A. Ammar, L. Naoufal, B. Azam, G.W. Dennis, A. David, Lightfoot Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts, Molecules 15 (2010) 7313–7352.
[12] B.Aliakbarian A.A.Casazza, S. Mantegna, G. Cravotto, P. Perego, Extraction of phenolics from Vitis vinifera wastes using non-conventional techniques, J. Food Eng. 100 (1) (2010) 50–55.
[13] F.S. Taha, G.F. Mohamed, S.H. Mohamed, S.S. Mohamed, M.M. Kamil, Optimization of the extraction of total phenolic compounds from sunflower meal and evaluation of the bioactivities of chosen extracts, Am. J. Food. Tech 6 (12) (2011) 1002–1020.
[14] M.E. Balogun, J.O. Oji, E.E. Besong, A.A. Ajah, E.M. Michael, Anti-ulcer activity of aqueous leaf extract of Nauclea latifolia (rubiaceae) on indomethacin-induced gastric ulcer in rats, Afr. J. Biotechnol. 12 (32) (2013) 5080–5086.
[15] A.M. El-Mahmood, J.H. Doughari, F.J. Chanji, In vitro antibacterial activities of crude extracts of Nauclea latifolia and Daniella Oliveri, Sci. Res. Essay 3 (3) (2008) 102–105.
[16] M.O. Odey, J.T. Johnson, I.A. Iwara, B. Gauje, N.S. Akpan, U.O. Luke, A.E. Robert, K.M. Ukpong, Effect of antihypertensive treatment with root and stem bark extracts of Nauclea latifolia on serum lipid profile, G. J. P&A Sc Tech. 2 (4) (2012) 78–84.
[17] Z.A. Nworgu, D.N. Onwukaeme, A.J. Afolayan, F.C. Ameachina, B.A. Ayinde, Preliminary studies of blood pressure lowering effect of Nauclea latifolia in rats, Afri. J. Pharm. Pharmacol. 2 (2) (2008) 37–41.
[18] Y. Deeni, H. Hussain, Screening for antimicrobial activity and for alkaloids of Nauclea latifolia, J. Ethnopharmacol. 35 (1991) 91–96.
[19] J. Omale, H. Haruna, H. Ugbede, Antihypertensive effect of methanol extract of Napoleona imperialis (p. beauv) in adrenaline induced hypertensive albino rats, Am. J. Trop. Med. Publ. Health 1 (1) (2011) 11–21.
[20] C.O. Nwaehujor, E.O. Onyenweaku, I.I. Ezeigbo, O.V. Asuzu, Evaluation of the hepato-protective potential of Sarcocephalus latifolius leaf methanol extracts in paracetamol-induced liver damage of mice, Comparat. Clin. Pathol. 25 (5) (2016) 1053–1059.
[21] H. Kadiri, E. Adegor, S. Asagba, Effect of aqueous Nauclea pobeguinii leaf extract on rats induced with hepatic injury, Res. J. Med. Plant. 1 (4) (2007) 139–143.
[22] S.C. Udeh, I.I. Madubunyi, Hepatoprotective activities of methanolic extract of Nauclea latifolia, Agro-Sci. 7 (1) (2008) 72–77.
[23] Y.K. Adamu, A.S. Aderonke, E.O. Ogbadoyi, Therapeutic effects of *Annona senegalensis* Pers stem bark extracts in experimental African trypanosomiasis, Int. J. Health Res. 3 (1) (2010) 45–49.
[24] J.P. McDonald, et al., DNA polymerase iota and related rad30-like enzymes, Philos Trans. R Soc. Lond. B. Biol. Sci. 356 (2001) (1405) 53–60.
[25] I.C. Ferreira, F.R. Aires, E. Barreira, L.M. Estevinho, Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract, Food Chem. 114 (2009) 1438–1443.
[26] A. Sofowora, in: The State of Medicinal Plants in Nigeria, 1st Ed, University of Ibadan Press, Nigeria, 1993 pp, pp. 206–210.
[27] J.V. Dacie, S.M. Lewis, in: Practical Haematology, 7th ed., Churchil Livingstone, Edinburgh, 1991, pp. 54–79.
[28] M.M. Ashraf, A.H. Sharaf, H. Farrag, M.F. Hany, Protective effects of vitamin c on hematological and biochemical parameters of intoxicated male albino rats with lead and cadmium, Middle East J. Appl. Sci. 07 (01) (2017) 57–67.
[29] Organisation for Economic Co-operation and Development, Guideline for the test of chemicals. guidance document on acute oral toxicity. Enviromental health and safety monograph series on testing and assessment, OECD/OCDE 425 (2008) 2.
[30] B.S. Okediran, K.T. Biobaku, F.H. Olaifa, A.J. Atata, Haematological and antioxidant enzyme response to Lead toxicity in male Wistar rats, Ceylon J. Sci. 46 (2) (2017) 31–37.
[31] F.A Suleiman, A. Mary, S. Muftau, U.K. Mohammed, Hematological changes induced by subchronic co-administration of chlorpyrifos and lead in Wistar rats: Alleviating effect of vitamin C, Der Pharmacia Sinica 2 (2) (2011) 276–284.
[32] A.A. Saeed, Hematobiochemical changes induced by lead intoxication in male and female albinomice, Natl. J. Physiol. Pharm. Pharmacol. 6 (1) (2016) 46–51.
[33] E.O. Adewoye, A.T. Salami, B.O. Emikpe, Effect of methanolic extract of chrysophyllum albidum bark on hematological indices in mice with experimental hemorrhagic anemia, Afr. J. Biomed. (2012) 309–314.
[34] T.M. Mahdy, T. Giorgi, F. Adewole, I. Ernest, M. Idoko, N. Matey, N. Ozele, S. Oladipo, M. Sunday, Effect of Moringa oleifera, activated carbon and wood charcoal on biochemical and hematological parameters of Wistar rats exposed to lead acetate, Med. Weter 68 (2012) 101–106.
[35] O. Ladokun, O. Matthew, A. Oluwatosin, Comparative study on the effects of aqueous extracts of viscum album (mistletoe) from three host plants on hematological parameters in albino rats, Afr. Health Sci. 15 (2) (2015) 607–612.
[36] A.U. Ekanem, H.D. Kwari, S.H. Garba, H.A. Salami, Effect of lead acetate on spleen and blood parameters in albino rats, J. Dent. Med. Sci. 14 (2015) 43–49.
[37] M.S. Zaki, S. Moustafa, H. Rashad, N. Sharaf, Assessment of the hazardous effect of lead pollution on Oreochromis niloticus, including hematological, biochemical and immunological parameters, Am. Euras. J. Agric. Environ. Sci. 3 (2008) 91–95.
[38] F.I. Akaneme, Identification and preliminary phytochemical analysis of herbs that can arrest threatened miscarriage in Orba and Nsukka towns of Enugu State, Afri. J. Biotechnol. 7 (1) (2008) 6.
[39] E.A. Aticka, The effect of lead on lung histology of albino mice Mus musculus, Rafid. J. Sci. 20 (3) (2009) 29–36.
[40] R.A Goyer, T.W. Clarkson, Toxic Effects of Metals, in: C.D. Klaasen (Ed.), Casarett and Doullis Toxicology. The Basic Science of Poisons, 6th Ed., McGraw-Hill, New York, 2001, pp. 861–867.