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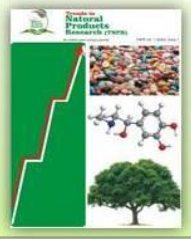

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis and Toxicological Evaluation of Methanol Leaf Extract of *Napoleona vogelii* Hook (Lecythidaceae)

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Keywords: Phytochemical constituents, toxicity, hematological indices, biochemical parameters, histology.

Abstract: The leaves of *Napoleona vogelii* Hook (Lecythidaceae) are used in ethnomedicine as antitussive, anti-asthmatic, anti-ulcer, hypolipidemic, anti-diabetic, and antimicrobial. The aim of this study was to identify the compounds present in the methanol leaf extract and to evaluate its toxicological profile. The constituents were identified using gas chromatography-mass spectrometry (GC-MS). Oral acute toxicity was evaluated in rats. Doses of 250, 500, and 1000 mg/kg/day were administered to groups of rats for 21 consecutive days after which weight, hematological, biochemical, and histological markers of toxicity were evaluated. The GC-MS analysis showed 25 peaks corresponding to 25 different compounds. The oral median lethal dose (LD_{50}) was greater than 5000 mg/kg. Following repeated administration for 21 days, there were no significant effects on weight indices, hematological parameters, serum proteins, bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase and lipids. There was a dose-dependent significant reduction in the concentrations of bicarbonate. Histologically, no abnormality except vasodilatation was observed in the heart, lungs, liver, kidneys, brain, lungs, spleen and liver of extract-treated rats. Knowledge of the constituents provides a scientific basis for some of the ethnomedicinal uses while the toxicological results suggest that the extract is relatively safe.

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DOI: 10.48245/tnpr-2734391.2022.3.101
Page No.: 1-22
Volume: 3, Issue 1, 2022
Trends in Natural Products Research

INTRODUCTION

About 2,000 plants species have the potential for being used as medicinal plants (Bodeker *et al*., 2005). Over three-quarters of the world population rely mainly on plants and materials derived from them for their healthcare needs (WHO, 2019). Herbal medicines are gaining popularity in developed and developing countries alike (Oyebode *et al*., 2016). Knowledge of the constituents and safety profile of herbal medicinal products is essential for drug development and standardization (WHO, 2004). *Napoleona vogelii* Hook and Planch (Lecythidacea**e**) is an angiosperm of the order Esricale (Odugbemi, 2006). In West Africa, its distribution extends from Sierra Leone to Nigeria along the seashore and it is used in making wooden poles, wraps, chewing sticks and mats (Keay *et al*., 1994). It is used in ethnomedicine for the treatment of several disease conditions including cough and asthma (Akah *et al*., 2007). There are scientific reports on the potentials of the plant as an anti-ulcer (Akah *et al*., 2007); a medicine for wound healing (Enye *et al*., 2013; Adiele *et al*., 2014); a hypolipidemic, an antidiabetic (Owolabi *et al*., 2014), and a medicine for microbial infections (Eze and Unachukwu, 2015). The anti-asthmatic and antitussive properties have also been evaluated (Adejayan *et al*., 2018).

Although no compounds have been identified in or isolated from the leaves of *N. vogelii*, the presence of secondary metabolites such as saponins, cardiac glycosides, anthraquinone, flavonoids, terpenoids phenols, and hydrogen cyanide have been reported (Igidi and Edene, 2014; Adejayan *et al*., 2018). Knowledge of the compounds in the leaves may provide scientific explanations for some of the ethnomedicinal uses and may also provide templates for the development of new therapeutic molecules.

Herbalists in Nigeria often extract the dry leaves in alcohol for oral administration and some pharmacological properties have been investigated using the methanol leaf extract (Adejayan *et al*., 2018). Although the leaf extract has been used for various medicinal purposes, there seems to be lack of information on the safety profile after repeated daily administration. Some of the diseases such as asthma for which the leaf extract is used in ethnomedicine require repeated administration over a long period. In the present study, we sought to identify the compounds in the methanol leaf extract and evaluate the toxicological profile of the extract in rats.

MATERIALS AND METHODS

Plant material and extraction

Fresh leaves of *N. vogelii* were collected from Nsukka in Enugu State, Nigeria. The plant was authenticated at the International Centre for Ethnomedicine and Drug Development, Nsukka where an herbarium voucher (Reference: Inter CEDD/507) was deposited. The extraction process has been described by Adejayan *et al*. (2018). The leaves were dried under shade and then powdered using a mill. A quantity (1.6 kg) of the powdered material was macerated in 6.5 L of methanol for 72 h with intermittent stirring. The mixture was filtered and the filtrate concentrated to dryness using a hot air oven at 40℃ over 3 days (yield = 6.10 % w/w). The dried extract was stored in an amber-colored bottle at 4℃.

Experimental animals

Adult male and female Wistar rats weighing between 175±10 g (mean ± SD) was sourced from the animal house, Department of Pharmacology & Toxicology, University of Benin, Benin City, Nigeria. The male rats were kept in separate cages from the females but all rats were exposed to natural environmental temperature and lighting conditions. The rats were fed on grower mash (Obby Anointed Agric Services, Benin City) with free access to tap water. All experiments were carried out in accordance with the Institute for Laboratory Animal Research Guidelines for the Care and Use of Laboratory Animals (National Research Council, 2011). In addition, approval (Reference: EC/018/12) was obtained from institutional ethics committee.

Gas chromatography-Mass Spectrometry (GC-MS) Analysis

The extract (0.1 g) was dissolved in 10 mL of 70 % methanol and was allowed to stand for 1 to 2 h in a closed test tube. The extracted sample was decanted, centrifuged and filtered using a micron filter into a 5 mL sample bottle. Analysis of the methanol extract was done using a gas chromatography instrument (model 7890A, Agilent USA) (Olivia *et al*., 2021). The instrument was hyphenated to a mass spectrophotometer (model 5975C) having a triple axis detector equipped with a 10 µL syringe. Helium served as the carrier gas. All chromatographic separations were done using capillary columns with the following specification: 30 m length; 0.2 µm internal diameter; 250 µm thickness; and 5 % phenylmethyl siloxane was used for treatment. Other conditions of the GC-MS include: ion source temperature of (ED) 250℃; interface temperature of 300℃; pressure of 16.2 psia; out time of 1.8 mm; 1 µL injector mode with split ratio 1:50 with injection temperature of 300℃. The column temperature was started at 35℃ for 5 min and changed to 150℃ at the rate of 4℃ per min. The temperature was raised to 250℃ at the rate of 20℃ per min and was held at that temperature for 5 min. The flow rate was set at 1.5

mL/min and total elution time was 47.5 min. The system was controlled for data acquisition using the MS solution software provided by the supplier of the instrument. The compounds were identified by name, molecular formula, and molecular weight by comparing the mass spectra obtained with those of standard spectra from National Institute of Standards & Technology (NIST) library.

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Chemicals

Commercial kits for the assay of enzymes and lipids were manufactured by Randox Laboratories (UK). Methanol for the extraction process was manufactured by Sigma-Aldrich (Germany). All other chemicals and reagents were obtained from reputable manufacturers and solutions were prepared fresh each day. Distilled water was used to reconstitute the extract before administration by the oral route.

Oral acute toxicity test

Oral median lethal dose (LD_{50}) was evaluated using the Lorke (1983) method. In the first phase, three groups of rats (n=3) were administered 10, 100, and 1000 mg/kg respectively. The absence of death within 24 h necessitated the second phase that involved three groups (n=1) administered 1600, 2900, and 5000 mg/kg respectively. In both phases, the animals were observed constantly for the first hour, intermittently over the next four hours and then after 24 h in order to monitor mortality as well as other symptoms of toxicity. All animals were kept under observation for another 14 days.

Oral sub-acute toxicity evaluation

Twenty-four animals were randomly allotted into four groups of six rats per group (males = females). Group I received 10 mL/kg of distilled water; groups II, III, and IV received 250, 500, and 1000 mg/kg of the extract respectively once daily for 21 days. The rats were weighed weekly and were observed daily for behavior, mortality, and other symptoms of toxicity. On the 21st day, all animals were sacrificed under chloroform anesthesia one hour after the day's treatment. Blood samples were withdrawn from the abdominal aorta with a 21G needle mounted on a 10 mL syringe (Agary Pharmaceutical Ltd, Nigeria) into lithium-heparin and EDTA sample bottles (BD Vacutsiners, BD-Plymouth, UK) and processed for biochemical and hematological assays.

Internal organs including the heart, brain, lungs, liver, kidney, and spleen were collected from randomly selected rats in each of the groups such that a male and a female were included. The organs were stored in 20 mL universal sample bottles (Axiom Zhanjiang Gong Jong Medical Technology Co. Ltd., China) containing 10 % formalin and processed for histopathological evaluation.

Evaluation of Weight Indices

Body weight changes

The mean weekly weights (days 7, 14, 21) of the rats in each group were compared with their initial weights (day 0).

Relative organ weight

Prior to sacrificing, each rat was weighed, after which the organs (heart, lungs, spleen, kidney, liver, and brain) were excised and weighed. The relative organ weight was calculated as follows:

$$Relative\ Organ\ Weight\ \ (\%) = \frac{Weight\ of\ Organ\ (g)}{Weight\ of\ Rat\ on\ Day\ of\ Sacrifice\ (g)} \times 100$$

Evaluation of Hematological Indices

Blood samples collected into ethylenediamine tetra acetic acid (EDTA) bottles were used for the evaluation of red blood cell count (RBC), packed cell volume (PCV), hemoglobin concentration (Hb), platelet count (PLT), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and total white blood cell counts (WBC) and the differentials (granulocytes, lymphocytes and monocytes). The samples were analyzed using an automated hematology system (Diatron Abacus Junior Hematology Analyzer, China) (Akhigbemen *et al*., 2018).

Biochemical Assays

Blood samples in plain bottles were allowed to clot at room temperature for 4 h before centrifugation using a Hettich® centrifuge (Rototix 32A, Germany) at 4000 rpm for 10 min. The sera obtained were used for evaluation of biochemical parameters including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and lipid profile using standard diagnostic test kits (Randox laboratories, UK) on Automated Clinical System (VIS-7220G, Biotech Engineering Management Company Limited, UK; Analyzer ISE 4000 SFRI, France) following the manufacturer's instructions. Total bilirubin (TB) and direct bilirubin (DB) was determined using Jendrassik-Grof method (Spencer and Price, 1977). Urea was assayed using modified diacetyl monoamine method (Marsh *et al*., 1965), while creatinine was determined by the Jaffe's method (Chawla, 1999). Ion selective electrode machine was used for the assay of sodium, potassium, chloride and bicarbonate using the method described by (Burnett *et al*., 2000).

Histology

The hearts, lungs, spleens, kidneys, livers and brains were processed by fixing in 10 % formalin (Pyrex Scientific Co. Nigeria Ltd) and embedded in paraffin. Afterwards the tissues were cut into sections of 5 µm thick using a microtome. The sections were placed on slides, de-paraffinized by running them through xylene, alcohol and water in sequence. This was followed by staining of the slides with hematoxylin and eosin (H&E). The specimens were mounted on the slides with coverslip, dehydrated with alcohol and viewed under a light microscope (Olympus XSZ-107BN, China) at a magnification of x100. An attached camera (Eakins 1080P, China) was used to take photographs of the slides.

Statistical analysis

Data are expressed as mean ± SEM (standard error of mean). The data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* using GraphPad software (GraphPad Instat Inc., USA). Differences were considered significant at $p < 0.05$.

RESULTS

Phytoconstituents of the Extract

The GC-MS chromatogram of the methanol leaf extract of *N. vogelii* with characteristic retention time and peak wavelength of maximum absorption for each compound is shown in Figure 1. The 25 identified constituents including molecular formulas are given in Table 1.

Oral Acute Toxicity of the Extract

The extract did not cause death or any observable symptom except sedation at doses of 1000 mg/kg and above (Table 2). The oral LD_{50} was therefore estimated to be greater than the maximum dose of 5000 mg/kg used in the study.

Oral Sub-acute Toxicity of the Extract

Effect on weight indices

Oral treatment with doses of 250, 500, and 1000 mg/kg/day of the extract for 21 days did not significantly alter whole body weights (Table 3) and the relative weight indices of the heart, lungs, spleen, liver, kidney, and brain of rats when compared with the control (Table 4).

Effect on hematological parameters

Treatment with 250, 500, and 1000 mg/kg/day of the extract did not significantly alter red blood cell parameters (count, hemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin concentration) and platelet count when compared with values from control rats (Table 5). Also, the extract did not significantly alter white blood cell count and its differentials (granulocytes, lymphocytes, and monocytes) when compared with the values from control rats (Table 6).

Effect on plasma electrolytes, urea, and creatinine

Table 7 shows that daily oral treatment with the extract caused a dose-dependent significant reduction in plasma bicarbonate ion concentration corresponding to $p < 0.03$ (250 mg/kg), $p < 0.02$ (500 mg/kg) and $p < 0.005$ (1000 mg/kg). The values for Na^+, K^+, Cl^- creatinine, and urea were comparable across the groups.

Effect on plasma lipids

There were no significant differences in the lipid parameters: total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL) and triglycerides (Table 8).

Effect on plasma proteins and bilirubin

Table 9 shows that there were no significant differences in the plasma concentration of proteins and bilirubin in extract-treated rats when compared with the control.

Effect on plasma enzymes

Daily oral treatment of rats with the extract for 21 consecutive days did not cause significant changes in the plasma levels of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) (Figures 2 to 4).

Effects on the histology of selected organs

In the heart (Figure 5), there were no major histological changes except active congestion in the interstitial space and vasodilatation that occurred at all doses (resulting in increased blood flow) when compared with the control. The photomicrographs of the lungs are shown in Figure 6. There were no major architectural changes in the bronchial artery and alveoli of extract-treated rats when compared to the control. However, at 250 mg/kg/day there was florid lymphoid activation, mild active congestion and vasodilatation. At 500 mg/kg/day there was florid lymphoid activation, bronchodilation, active interstitial congestion and vasodilatation. At 1000 mg/kg/day, there was active interstitial congestion, florid lymphoid activation and vasodilatation.

In the spleen, photomicrographs (Figure 7) show that treatment did not cause any histological changes when compared with the control except for follicular activation and sinus histiocytosis that occurred at all the doses.

Although there were no histological changes on the bile duct, hepatocytes and portal vein in the liver of rats when compared with the control, there was sinusoidal Kupffer cell activation at the dose of 250 and 500 mg/kg/day. At the dose of 1000 mg/kg/day, there was active vascular congestion (Figure 8).

In the kidney (Figure 9), doses of the extract did not cause any observable effect on the glomerulus and tubules but caused active interstitial congestion and vasodilatation when compared to the control.

Nothing abnormal was observed on the brain (molecular layer, Purkinje layer, granular layer, white and grey matter) except for mild active vascular congestion and vasodilatation (Figure 10).

DISCUSSION

The present study has revealed the compounds in the methanol leaf extract of *N. vogelii*. It has also shown that the extract is relatively safe when administered by the oral route. Out of the 25 compounds identified, some (e.g., compounds 11 and 21) appear novel while many others (e.g., compounds 5, 6, 8 which are derivatives of naphthalene; 12, 15) have been found in other plants. Some of the compounds have been associated with important biological activities seen in the leaf extract. For example, scoparone (compound 12) a coumarin, is known to have many beneficial therapeutic properties including antiatherogenic (Chen *et al*., 1994), vasodilator effects (Huang *et al*., 1992), ability to inhibit STAT 3-induced vascular smooth muscle proliferation (Park *et al*., 2015), and anti-inflammatory property (Liu *et al*., 2019) among others. The presence of protionamide (compound 19), an antitubercular drug (Scardigi *et al*., 2016) may explain the reported antimicrobial action of the leaf extract (Eze and Unachukwu, 2015). Similarly, 2-mercaptophenothiazine (compound 20) is a phenothiazine derivative and may therefore underscore the antimicrobial, anticancer, antihistaminic and smooth muscle relaxant properties that have been associated with the extract (Varga *et al*., 2017). These compounds may be exploited in drug development aside from providing scientific basis for the ethnomedicinal uses of the plant.

An oral LD_{50} value higher than 5000 mg/kg suggests that the extract is relatively safe (OECD, 2001). Repeated exposure of rodents to substances often results in alteration of weight indices which may be suggestive of toxicity (Raza *et al*., 2002; Teo *et al*., 2002; Ozolua *et al*., 2010). The absence of significant changes in weight indices in all the extract-treated groups suggests that the extract may not have caused deleterious effects that could have affected the weight parameters.

Changes in the hematological parameters in animals may have implications for human toxicity (Ashafa *et al*., 2012). For example, a decrease in red blood cell count is indicative of anemia due to hemolysis, hemorrhage or the production of immature reticulocytes usually associated with iron deficiency (Arika *et al*., 2016) while a marked change in WBC and its differentials in the blood may suggest a change in the immunological status of the animals (Rashid, 2013). In the present study, the extract seems not to have had any adverse effect on the hematological parameters.

Nephrotoxicity which manifests as oliguria and alteration in the plasma levels of electrolytes, urea and creatinine is one of the most common problems associated with repeated exposure to a toxicant (Bazari, 2007). In the present study, only bicarbonate concentration was significantly altered as a result of treatment with the extract. Bicarbonate ions act as buffer to maintain the serum pH within normal range. Although a decrease in concentration of bicarbonate ions may suggest metabolic acidosis (Meert *et al*., 2007), the absence of concomitant alterations in serum concentrations of Na^+, K^+, Cl^-, creatinine and urea among the treated rats does not support the presence of nephrotoxicity.

Lipid profile indices are useful in monitoring the state of the cardiovascular system (Flegal *et al*., 2002). Elevated level of triglycerides and LDL-C is a predisposing factor to atherosclerosis and its cardiovascular-related sequelae (Rishi *et al*., 2016; Nicholls *et al*., 2018; George *et al*., 2021). The present study which has shown that the serum lipids are not significantly altered contradicts an earlier study by Owolabi *et al*. (2014) who observed derangement in lipids in alloxan-induced diabetic rats administered the extract at the doses of 100, 200, 400 mg/kg/day for 14 days.

Serum enzymes and other proteins have been used to evaluate the integrity of internal organs (Busher, 1990). For example, the health status of the liver can be evaluated using serum levels of proteins, transaminases, gamma-glutamyl transferase, and alkaline phosphatase (Adeoye and Oyedepo, 2004). While low concentration of total proteins in serum may suggest liver, and/or kidney disorders or poor digestion and malabsorption, high levels of total protein may indicate chronic inflammation or liver infections (Lala *et al*., 2022). In the present study, no derangement was seen in the values of total proteins and or its constituents such as albumin and globulin. Although increased serum levels of AST could be diagnostic of liver, kidney, heart, and pancreatic injuries, increased ALT level has been regarded as a more specific diagnostic marker of liver injury (Ozer *et al*., 2008). Similarly, even though increase in serum levels of ALP is associated with liver, kidney and bone pathologies, it has a strong relationship

with biliary obstruction (Lowe *et al*., 2021). The absence of significant changes in the levels of these diagnostic enzymes in all the groups of rats treated with the extract suggests that harm may not have resulted from the treatment.

In the present study, biochemical findings seem to have been strengthened by histological observations. In the extract-treated animals, the histology of the heart was normal but there was increased luminal size of blood vessels which suggests vasodilatation that may have resulted in the active congestion of the interstitial space. Vasodilatation reflects enhanced tissue perfusion (Aliya, 2011). In the lungs, aside from bronchodilation which corroborates a previous report (Adejayan *et al*., 2018), there was lymphoid activation that may indicate a potential for immune boosting (Rashid, 2013). This property was also reflected in increased levels of histiocytes in the organs, and activation of Kupffer cells in the liver. Brain histology was essentially normal except for vasodilatation which may predispose to headaches and elevated intracranial pressure (Bednarczky *et al*., 2002). The extract did not affect the glomeruli and tubules but it caused vasodilatation and interstitial congestion which did not manifest in altered renal function as accentuated by normal serum electrolytes, creatinine and urea concentrations.

In conclusion, the methanol leaf extract contains bioactive compounds that may underscore the reported ethnomedicinal uses such as anti-asthmatic and antibacterial. This study has shown that the extract has an LD_{50} value that is greater than 5000 mg/kg and therefore seems safe for use. The bioactive molecules may serve as templates for newer drugs with better pharmacological profiles.

Acknowledgments

The authors are grateful to Mr. Collins Osaigbovo and the animal house staff of the Department of Pharmacology & Toxicology, University of Benin for their assistance. The technical support of the laboratory staff of Departments of Morbid Anatomy and Chemical Pathology, University of Benin, is acknowledged. The study was part of the final year Pharm D project dissertation by EIO at the University of Benin.

Author Contributions

EIO and AMA carried out the experiments; GIE carried out the histology; SMM supervised the biochemical assays; NTA supervised the GC-MS analysis; and RIO conceptualized the study and supervised all aspects. The statistical analysis and preparation of the manuscript was done by EIO, AMA and RIO. All the authors read and agreed to the contents of the manuscript before it was submitted.

Funding

No funding was received for conducting this study.

Conflict of Interest

The authors declare no conflict of interest.

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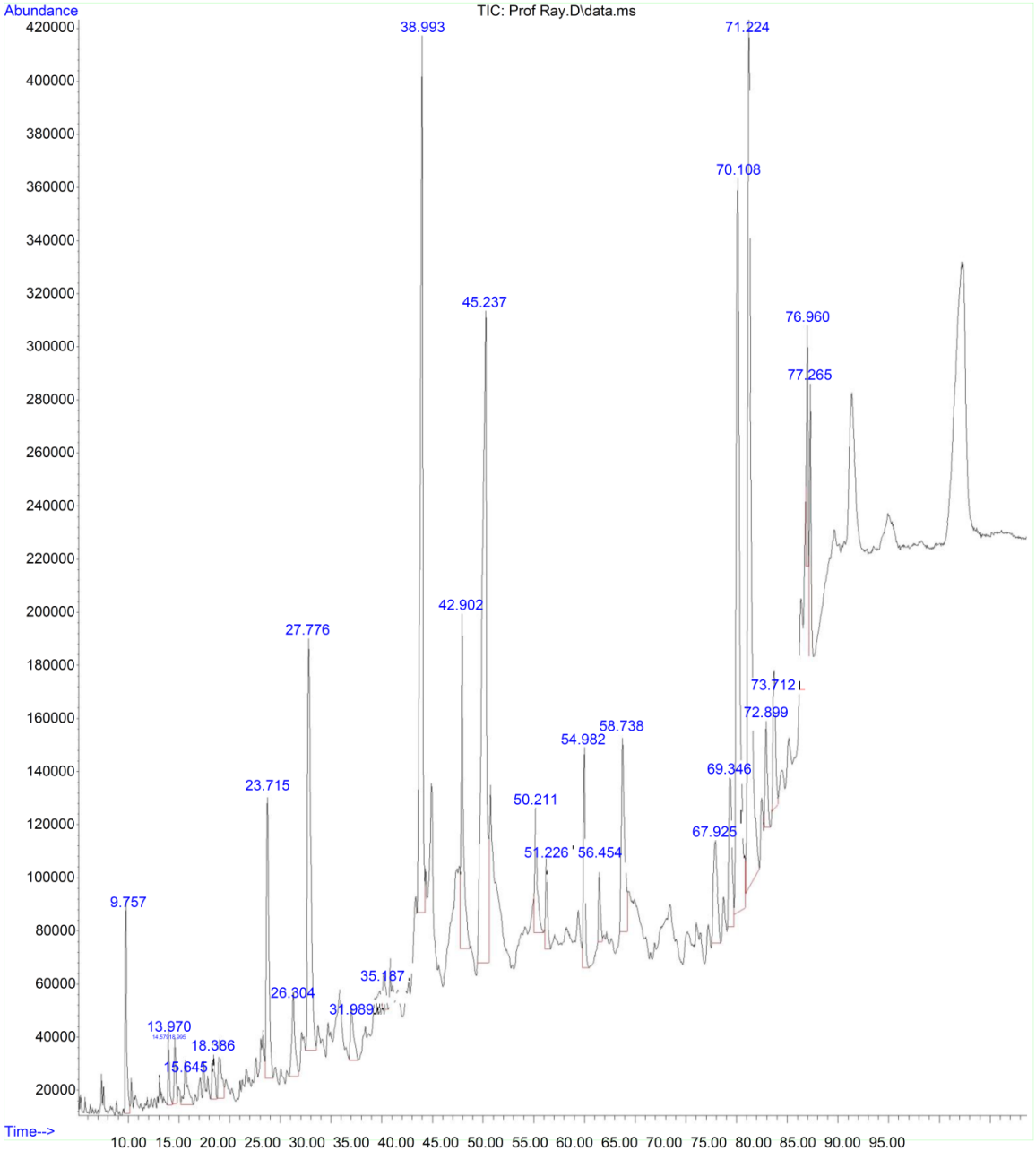

Figure 1: GC-MS spectra of *N. vogelii* methanol leaf extract showing the peaks for each of the identified compounds

Table 1: GC-MS identified constituents of *N. vogelii* methanol leaf extract

#	Name of Compound	Retention Time	Molecular Weight (g/mol)	Molecular Formula
1	Isothiazole 3-carboxamide	9.757	128.15	$C_4H_4N_2OS$
2	Methyl iodide	13.970	141.94	CH_3I
3	[1,2,3,4]Tetrathiine	14.579	154.297	$C_2H_2S_4$
4	Thiazolo[5,4-d]pyrimidine-7(4H)-thione, 5-methyl-	15.645	183.3	$C_6H_5N_3S_2$
5	Naphthalene, 1,6-dimethyl-	18.386	156.22	$C_{12}\ H_{12}$
6	Naphthalene, 1,5-dimethyl-	18.995	166.3	$C_{12}H_{22}$
7	Phenol, 2,5-bis(1,1-dimethylethyl	23.715	206.3239	$C_{14}H_{22}O$
8	Naphthalene, 2,3,6-trimethyl-	26.304	170.25	$C_{13}H_{14}$
9	Phenol, 2-[(trimethylsilyl)oxy]-	27.776	182.29	$C_9H_{14}O_2Si$
10	2-Hexa-2,4-dienylmalonic acid, dimethyl ester	31.989	212.24	$C_{11}H_{16}O_4$
11	1H-Pyrrolo[3,4-c] pyridine-1,3(2H)-dione, 2fluorophenyl)-	35.187	242.2	$C_{13}H_7FN_2O_2$
12	Scoparone (6,7-Dimethoxy-Coumarin)	38.993	206.19	$C_{11}H_{10}O_4$
13	2-Methoxy-1-[(E)-2-(1-naphthyl)-ethenyl]-diazene-1-oxide	42.902	221.11	$C_{13}H_5N_2O_2$
14	Acetamide, 2-cyano-N-[(ethylamino) carbonyl]-2-(methoxyimino)-	45.237	198.1793	$C_7H_{10}N_4O_3$
15	Benzoselenazole	50.211	182.09	C_7H_5NSe
16	(1-Naphthoxy) acetic acid	51.226	202.21	$C_{12}H_{10}O_3$
17	1,2,3-Trimethoxybenzene	54.982	168.19	$C_9H_{12}O_3$
18	Benzenamine, 2,6-dinitro-	56.454	183.12	$C_6H_5N_3O_4$
19	Protionamide	58.738	180.27	$C_9H_{12}N_2S$
20	2-Mercaptophenothiazine	67.925	245.4	$C_{13}H_{11}N_2S$
21	2-Isobutylsulfanyl-4,5,6-trimethyl -nicotinonitrile	69.346	234.36	$C_{13}H_{18}N_2S$
22	Furane-2-carboxaldehyde, 5-(2-meth yl-4-nitrophenyl)-	71.224	231.2	$C_{12}H_9NO_4$
23	Benzonitrile, 4-(2-cyano-2-phenylethenyl)	72.899	230.26	$C_{16}H_{10}N_2$
24	7H-Benz[de]anthracen-7-one	73.712	230.26	$C_{17}H_{10}O$
25	Phenol, 3,5-bis(1,1-dimethylethyl)	76.960	206.32	$C_{14}H_{22}O$

Table 2: Oral acute toxicity evaluation of methanol leaf extract of *N. vogelii* in rats

	Dose (mg/kg)	Writhing	Sedation	Grooming	Diarrhea	Convulsion	Death
Phase I	10	0/3	0/3	1/3	0/3	0/3	0/3
	100	0/3	0/3	3/3	0/3	0/3	0/3
	1000	0/3	3/3	3/3	0/3	0/3	0/3
Phase II	1600	0/1	1/1	1/1	0/1	0/1	0/1
	2900	0/1	1/1	1/1	0/1	0/1	0/1
	5000	0/1	1/1	1/1	0/1	0/1	0/1

Numerator = number of animals with observed effect; denominator = number of treated animals. LD_{50} > 5000 mg/kg (*per os*).

Table 3: Effect of 21-day oral administration of methanol leaf extract of *N. vogelii* on whole body weight of rats

	Mean Weight (g)			
	Day 1	Day 7	Day 14	Day 21
Control (10 ml/kg/day)	233.6±6.1	219.7±9.0	227.1±6.3	193.2±20.0
250 mg/kg/day	190.7±8.4	199.0±21.1	194.2±11.1	202.3±20.8
500 mg/kg/day	202.7±13.0	193.7±24.2	200.6±24.8	194.5±24.2
1000 mg/kg/day	220.3±18.1	220.3±31.7	240.2±29.7	224.5±27.4

Values are not significantly different. Data represent mean ± SEM; n=6 rats.

Table 4: Effect of 21-day oral treatment with methanol leaf extract of *N. vogelli* on relative organ weights of rats

Dose	LIVER (%)	KIDNEY (%)	SPLEEN (%)	HEART (%)	LUNGS (%)	BRAIN (%)
Control (10 ml/kg/day)	3.75±0.34	0.37±0.01	0.48±0.10	0.40±0.02	0.94±0.06	0.87±0.09
250 mg/kg/day	3.18±0.13	0.36±0.00	0.39±0.03	0.47±0.05	0.95±0.08	0.81±0.06
500 mg/kg/day	3.17±0.05	0.35±0.01	0.37±0.03	0.38±0.03	0.88±0.06	0.78±0.05
1000 mg/kg/day	3.54±0.14	0.34±0.02	0.39±0.03	0.43±0.01	0.96±0.05	0.78±0.05

Values are not significantly different. Data represent mean ± SEM; n=6 rats.

Table 5: Effect of 21-day oral treatment with methanol leaf extract of *N. vogelii* on red blood parameters and platelet count

Dose	RBC (x 10^6/µl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Hgb (g/dl)	PLT (x 10^3/µl)
Control (10 ml/kg/day)	7.0±0.3	41.7±1.4	59.5±1.1	17.7±0.8	29.8±1.3	12.5±0.7	666.5±10.3
250 mg/kg/day	7.4±0.5	41.8±2.1	57.0±1.6	17.0±0.8	29.9±1.1	12.4±0.5	539.8±73.5
500 mg/kg/day	7.5±0.3	42.6±1.3	57.2±1.1	18.5±1.0	32.3±1.2	13.7±0.4	586.0±44.1
1000 mg/kg/day	7.1±0.3	41.4±1.0	58.3±1.0	18.0±0.7	30.9±0.9	12.8±0.4	603.5±30.6

Values are not significantly different. Data represent mean ± SEM; n=6 rats. Hgb, hemoglobin; RBC, red blood cells; PLT, platelets; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; HCT, hematocrit.

Table 6: Effect of 21-day oral treatment with methanol leaf extract of *N. vogelii* on white blood cells and the differentials

Dose	WBC (x 10^3/µl)	LYM (x 10^3/µl)	MO (x10^3/µl)	GR (x10^3/µl)
Control (10 ml/kg/day)	7.50±3.34	6.23±1.00	0.65±0.22	0.78±0.24
250 mg/kg/day	5.98±0.96	5.31±1.06	0.23±0.03	0.45±0.13
500 mg/kg/day	5.75±0.51	4.88±0.61	0.35±0.09	0.51±0.18
1000 mg/kg/day	8.25±1.48	7.18±0.96	0.5±0.28	0.53±0.31

Values are not significantly different. Data represent mean ± SEM; n=6 rats. WBC, white blood cells; GR, granulocytes count; LYM, lymphocyte; MO, monocyte.

Table 7: Effect of 21-day oral treatment with methanol leaf extract of *N. vogelii* on plasma urea, creatinine and electrolytes.

Dose	Cr (g/dl)	Urea (g/dl)	Na^+ (mM)	K^+ (mM)	Cl^- (mM)	HCO_3^- (mM)
Control (10 ml/kg/day)	0.6±0.03	39.17±2.44	138.8±1.33	3.66±0.09	94.00±1.7	23.50±1.26
250 mg/kg/day	0.6±0.03	47.67±4.12	136.3±2.27	4.01±0.22	91.17±1.23	19.67±0.42*
500 mg/kg/day	0.52±0.05	47.33±3.64	141.5±0.76	4.10±0.18	100.3±0.92	19.21±0.81**
1000 mg/kg/day	0.5±0.03	39.83±2.58	135.0±2.20	4.18±0.09	94.67±2.77	18.83±0.75***

*P=0.025; **P=0.011; ***P=0.005 compared to control. Cr = creatinine. Data represents mean ± SEM; n=6 rats.

Table 8: Effect of 21-day oral treatment with methanol leaf extract *N. vogelii* on plasma lipids of rats

Dose	TC (mg/dl)	HDL (mg/dl)	TG (mg/dl)	LDL (mg/dl)
Control (10 ml/kg/day)	93.33±3.69	26.33±2.81	75.83±11.03	51.67±6.42
250 mg/kg/day	90.22±3.90	30.0±4.87	52.33±3.60	35.50±4.28
500 mg/kg/day	86.83±3.69	28.83±4.79	52.83±8.89	43.67±6.08
1000 mg/kg/day	93.50±5.64	22.33±2.42	66.50±8.54	57.83±7.46

Values are not significantly different. Data are represented as Mean ± SEM; n=6 rats. TC, Total cholesterol; HDL, High density Lipoprotein; TG, Triglyceride; LDL, Low density lipoprotein.

Table 9: Effect of 21-day oral treatment methanol leaf extract of *N. vogelii* on plasma protein and bilirubin of rats

Dose	CB (mg/dl)	TB (mg/dl)	ALB (mg/dl)	TP (mg/dl)	GB (mg/dl)
Control (10 ml/kg/day)	0.13±0.02	0.30±0.03	3.58±0.07	7.02±0.08	3.43±0.07
250 mg/kg/day	0.12±0.02	0.30±0.03	3.47±0.08	6.90±0.05	3.43±0.06
500 mg/kg/day	0.10±0.00	0.30±0.00	3.70±0.15	6.93±0.07	3.23±0.10
1000 mg/kg/day	0.12±0.02	0.27±0.04	3.66±0.03	7.13±0.062	3.46±0.05

Values are not significantly different. Data are presented as mean± SEM; n=6 rats. CB, Conjugated bilirubin; TB, Total Bilirubin; ALB, Albumin; TP, Total protein; GB, Globulin.

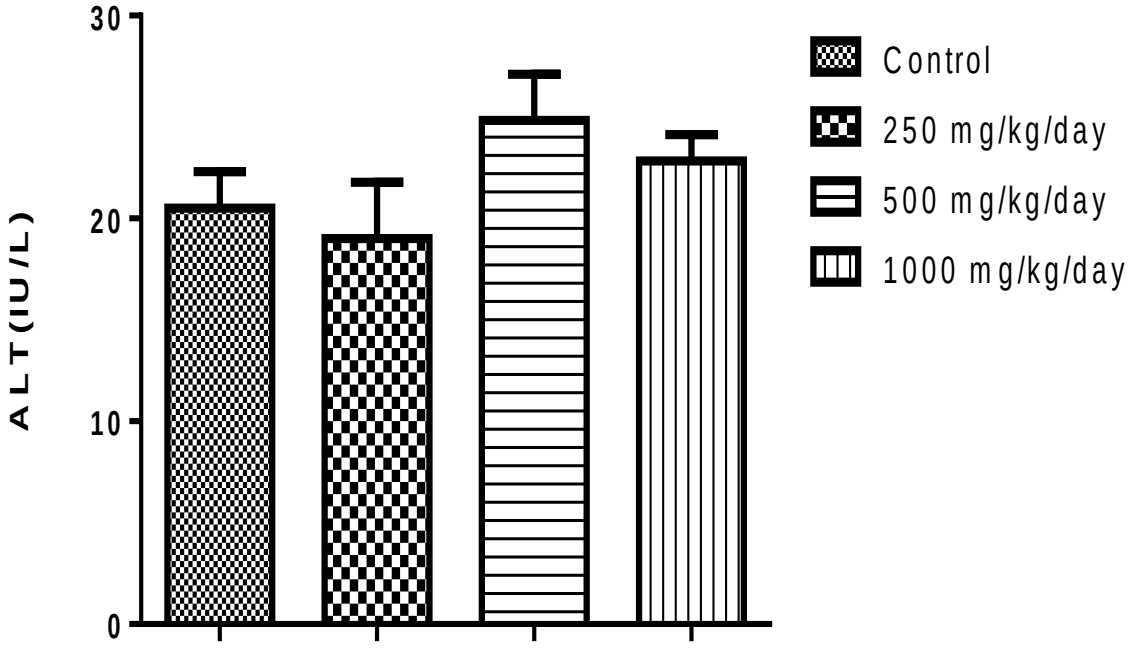

Figure 2: Effect of *N. vogelii* methanol leaf extract on alanine transaminase (ALT) following 21-day administration to rats. Values are not significantly different from the control. All values are expressed as mean ± SEM, n=6.

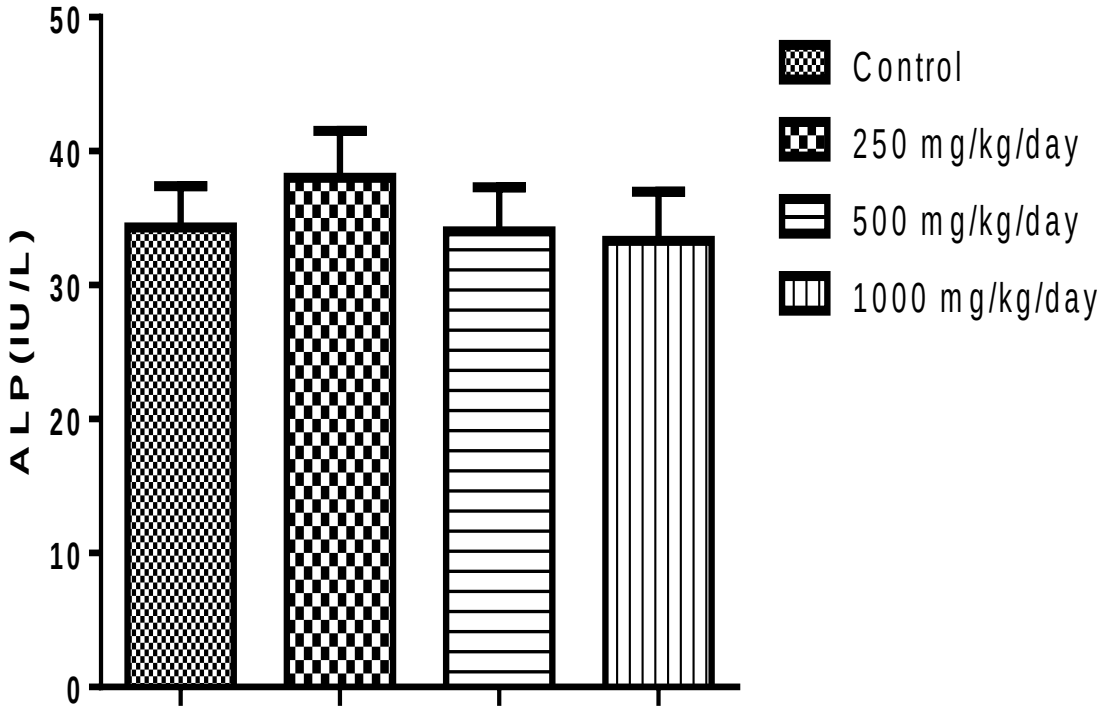

Figure 3: Effect of *N. vogelii* methanol leaf extract on alkaline phosphatase following 21-day administration to rats. Values are not significantly different from the control. All values are expressed as mean ± SEM, n=6.

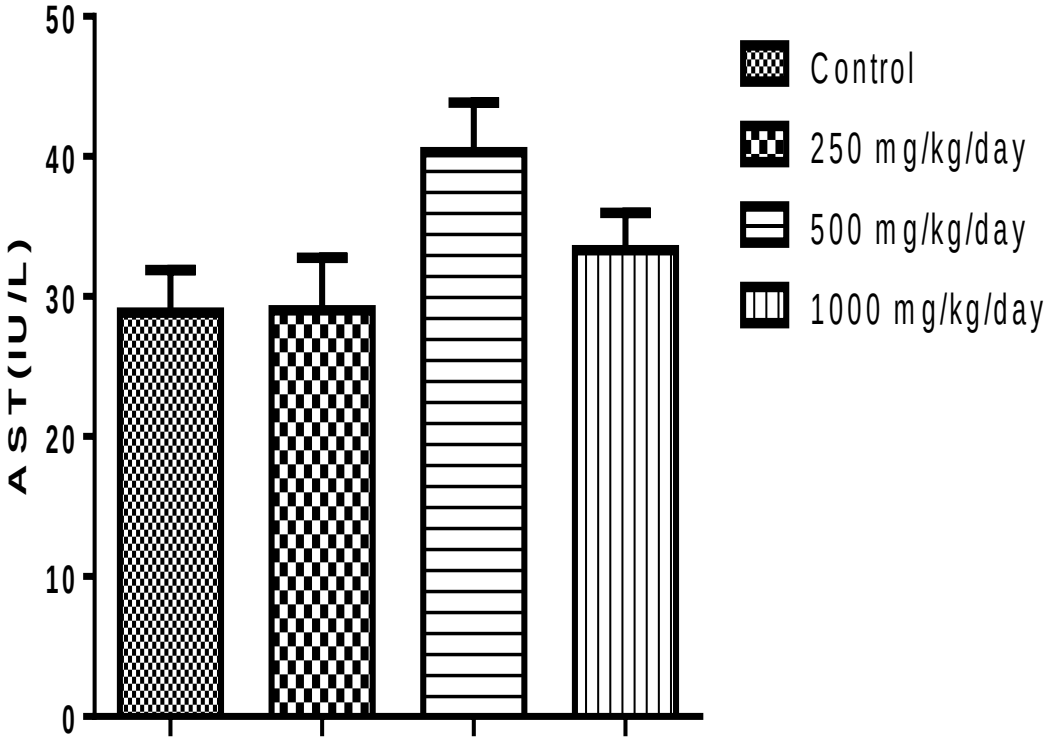

Figure 4: Effect of *N. vogelii* methanol leaf extract on aspartate transaminase following 21 days administration to rats. Values are not significantly different from the control. All values are expressed as mean ± SEM, n=6.

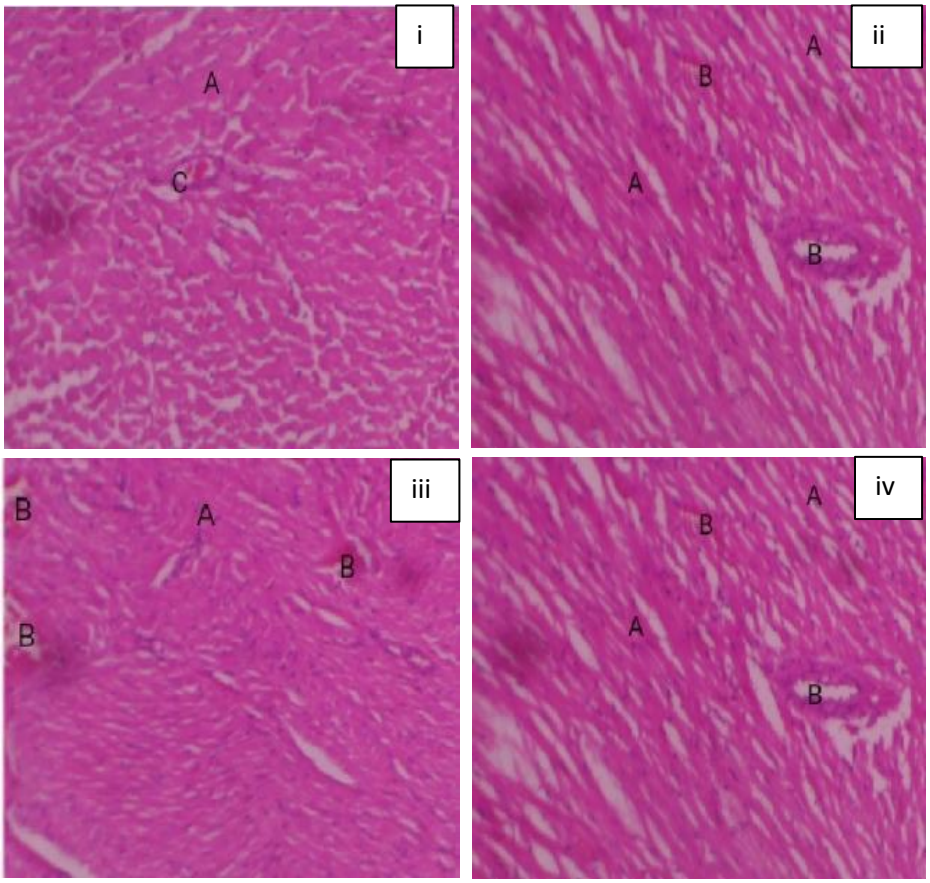

Figure 5: Representative photomicrographs of the heart of rats administered oral methanol leaf extract of *N. vogelii* for 21 days i: Control, ii: 250 mg/kg/day, iii: 500 mg/kg/day, iv: 1000 mg/kg/day. A - normal myocardial fibre; B - mild active congestion of interstitial space, vasodilatation; C - normal coronary artery. H & E, x 100

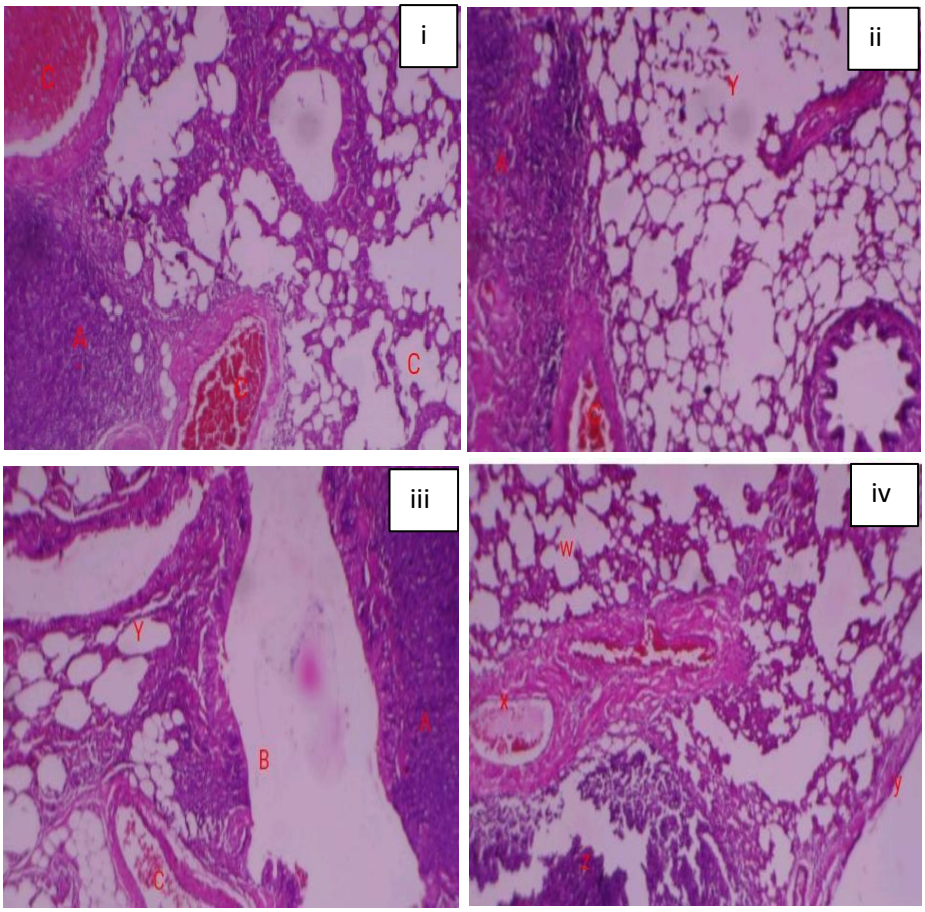

Figure 6: Representative photomicrographs of the lungs of rats orally administered methanol leaf extract of *N. vogelii* for 21 days i: Control, ii; 250 mg/kg/day, iii: 500 mg/kg/day, iv: 1000 mg/kg/day. W - normal bronchiole; X - normal bronchial artery; Y - normal alveoli; Z - bronchiolar lymphoid aggregates; A - florid lymphoid activation; B - bronchodilation; C - active interstitial congestion, vasodilation. H & E, × 100

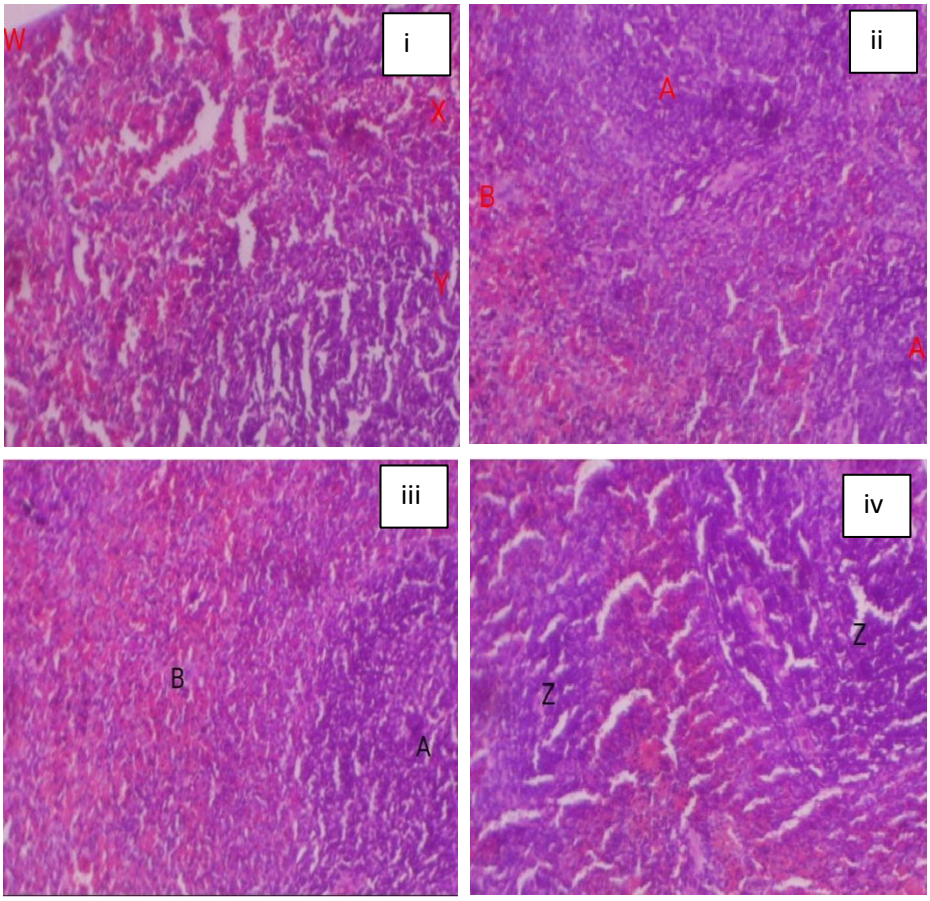

Figure 7: Representative photomicrographs of the spleen of rats orally administered methanol leaf extract of *N. vogelii* for 21 days. i: Control, ii; 250 mg/kg/day, iii: 500 mg/kg/day, iv: 1000 mg/kg/day. W - capsule; X - red pulp; Y - white pulp; A - follicular activation; B - sinus histiocytosis; Z - normal follicular architecture. H & E, × 100.

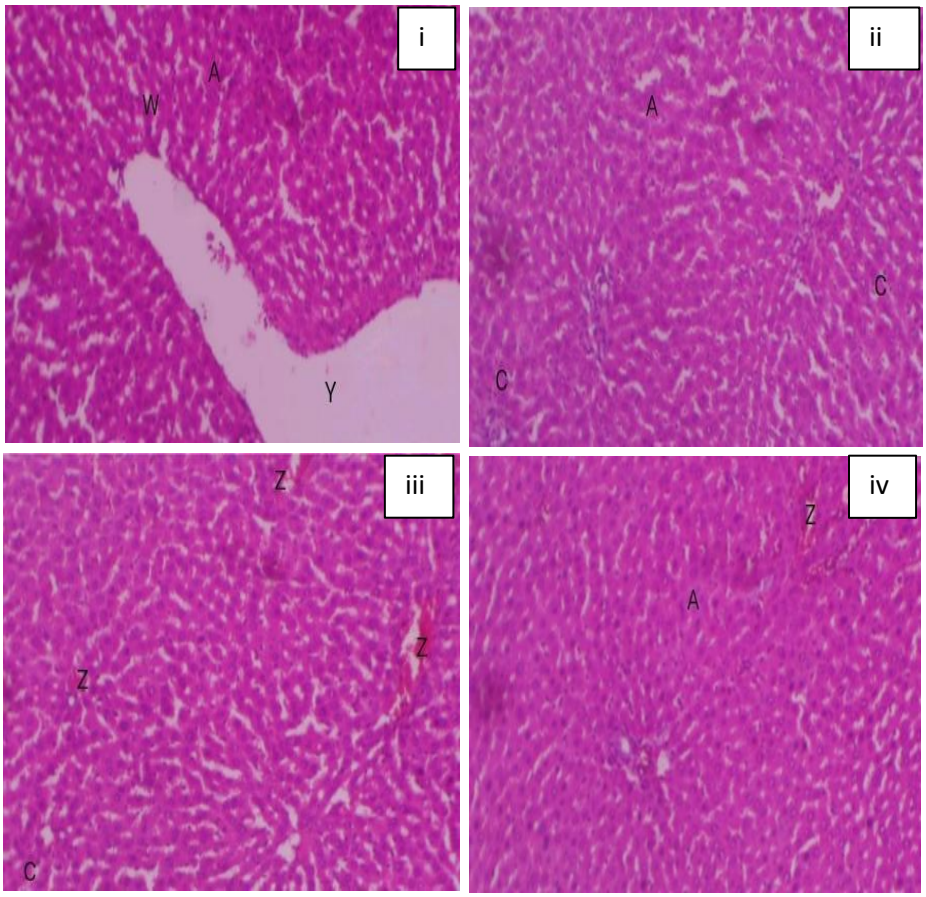

Figure 8: Representative photomicrographs of the liver of rat orally administered methanol leaf extract of *N. vogelii* for 21 days. i: Control, ii; 250 mg/kg/day, iii: 500 mg/kg/day, iv: 1000 mg/kg/day. Z - active vascular congestion; C - Kupffer cell activation. W - sinusoid; Y - portal vein; A - normal hepatocyte. H & E, × 100.

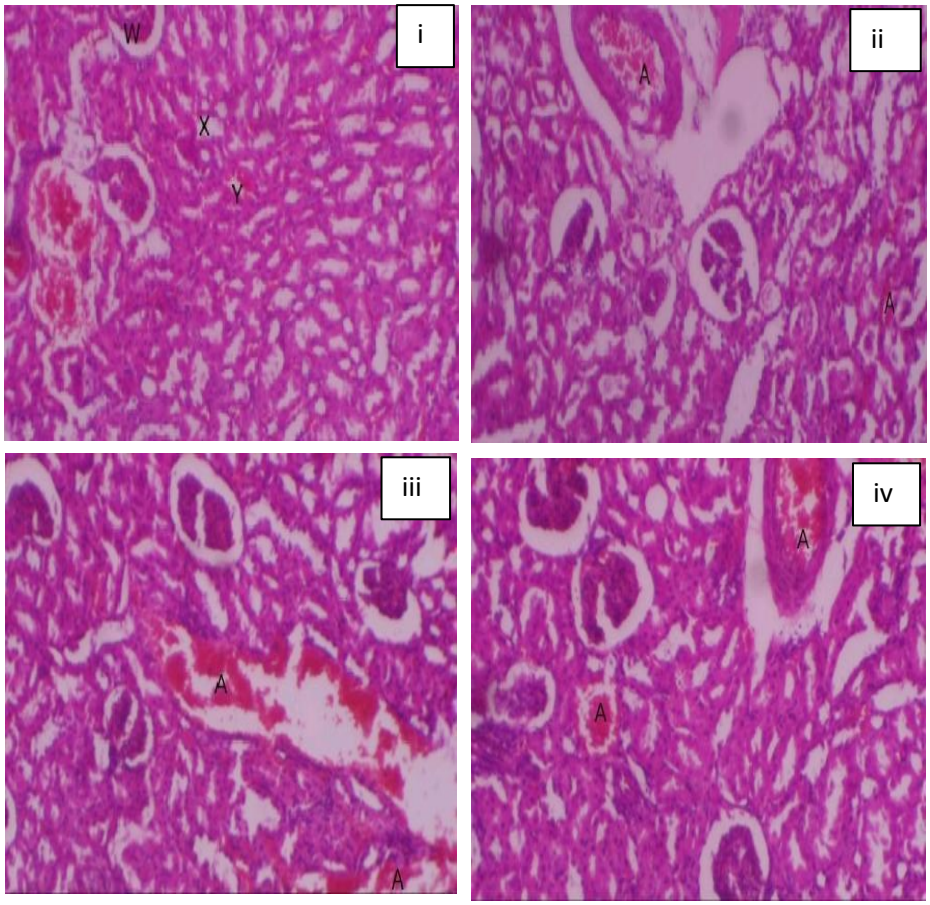

Figure 9: Representative photomicrographs of the kidney of rats administered methanol leaf extract of *N. vogelii* for 21 days i: Control, ii; 250 mg/kg/day, iii: 500 mg/kg/day, iv: 1000 mg/kg/day. W- glomerulus; X - tubules; Y - intertistial space; A - mild interstitial congestion. H & E, × 100

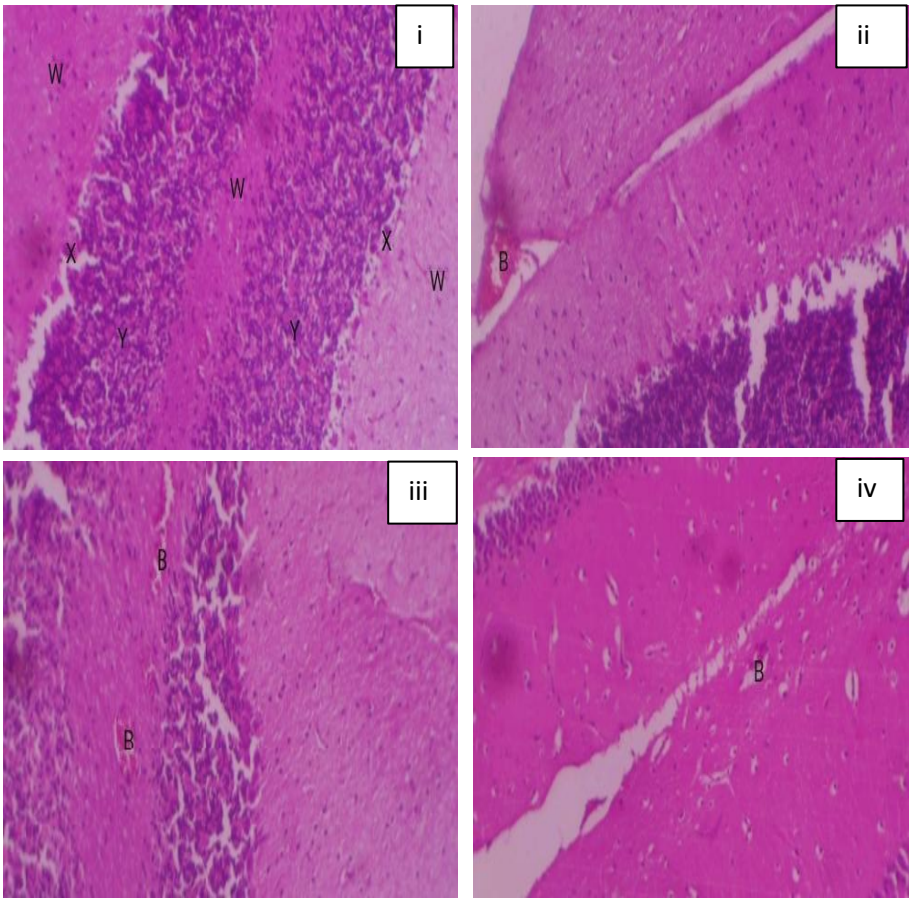

Figure 10: Representative photomicrographs of the brain of rats orally administered methanol leaf extract of *N. vogelii* for 21 days i: Control, ii; 250 mg/kg/day, iii: 500 mg/kg/day, iv: 1000 mg/kg/day. W - molecular layer; X - Purkinje layer; Y - granular layer; B - mild active vascular congestion. H & E × 100.

CITATION: Oheren EI, Akhigbemen AM, Eze GI, Momoh SM, Asogwa NT, Ozolua RI (2022). GC-MS Analysis and Toxicological Evaluation of Methanol Leaf Extract of *Napoleona vogelii* Hook (Lecythidaceae). Trend Nat Prod Res 3(1). 1-22. DOI: 10.48245/tnpr-2734391.2022.3.101