



***In vitro* Antibacterial Activity and *In vivo* Acute Toxicological Studies of *Nelsonia campestris* Aqueous Leaf Extract**

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Authors' contributions

This work was carried out in collaboration between all authors. Author HLM designed the study, wrote the protocol and supervised the work. Authors MBB and AF carried out all laboratories work and performed the statistical analysis. Author ASA managed the analyses of the study. Author AYK wrote the first draft of the manuscript. Author HAM managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate *In vitro* antibacterial activity and *in vivo* toxicological studies of aqueous extract of *Nelsonia campestris*.

Study Design: Experimental design.

Methodology: Standard laboratory procedures were used.

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Results and Discussion: The extract was highly active against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Klebsiella pneumonia* and moderately active against *Bacillus subtilis* and *Shigella dysenteriae* with inhibition diameters in the range of (20 mm-30 mm). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) recorded for the extract ranges between (80-90) mg/ml, and (70-90) mg/ml respectively. 100, 500, 1000, 2000 and 500 mg/kg bodyweight of the extract were orally administered to rats in their respective groups, while 0.5 ml of normal saline was administered to the rats in the control group for a period of one week. At the end of the experiment, the animals were anaesthetized under chloroform, and sacrificed. Blood samples were collected by jugular puncture and used for the analyses of biochemical and haematological parameters. The packed cell volume (PCV) in 100 mg/kg bodyweight group increased (38.0 ± 1.00) in reference to other groups. Red blood cell, and white blood cell also increased significantly ($p < 0.05$) when compared with the control group. Total protein decreased ($p > 0.05$) in all the treated groups in reference to the control. Activities of Aspartate transaminase (AST) increased in all groups in a dose dependent manner while that of alanine transaminase (ALT) and alkaline phosphatase (ALP) decreased but showing increase in the same dose dependent fashion. Triglyceride increased significantly ($p < 0.05$) in all groups, while cholesterol decreased in all groups. Urea increased significantly in a dose dependent manner when compared to the control group while creatinine decreased in the same way.

Conclusion: Aqueous extract of *Nelsonia campestris* has activity against the microbes resulting from the suppression of immunity by morbillivirus, but with mild toxicity to kidney, and liver.

Keywords: Antibacterial; hypolipidemic; haematological; biochemical; immunity.

1. INTRODUCTION

Plants contain a variety of chemical substances with varied physiological effects. They are reservoir of various chemical substances (phytochemicals) with potential therapeutic properties but with varied adverse effects. *Nelsonia campestris* is utilized by the Nupe speaking people of Niger state, Nigeria to treat measles caused by morbillivirus of the *Paramyxovirus* family, with concurrent development of other opportunistic infections such as pneumonia, conjunctivitis, diarrhoea, and infection of the otitis media. The primary benefit of using plant derived-medicine according to folkloric use is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits at affordable cost. But not all medicinal plants are safe for consumption as believed by the users. Some have been reported to be toxic, while some level of toxicity may arise through pesticide application or acquisition of some chemical compounds (e.g heavy metals) from the soil. The growing interest in herbal medicine therefore demands toxicity risk assessment of the various indigenous preparations used in the treatment of diseases. There is a strong believe that because herbal remedies are derived from nature, they are devoid of adverse effects sometimes associated with synthetic drugs [1]. The African continent is

one of the continents endowed with the richest biodiversity in plants used as herbs, food and for therapeutic purposes. One of such plants is *Nelsonia campestris* belonging to the family of *Acanthaceae*, and is a useful plant of tropical Africa, and Australia [2]. It grows on the margins of creeks, rivers, and down into the water but generally only near the edges. In semi-arid region of Northern Nigeria, it grows after the rain had ceased, and around every household thus making it accessible by the rural community. Other information provided by traditional practitioner in the part of Nigeria where the plant is used in treatment reveals that *Nelsonia campestris* is used in treatment of skin infections (including chicken pox), fevers, constipation and gastric ulcer, in addition to measles and lots of others. The patient is usually required to take a full cup of the plant decoction at least three times a day or take after meal instead of water.

According to Gupta [3] *Nelsonia campestris* at doses of 150, 300 and 500 mg/kg bodyweight was tested for its efficacy against paracetamol induced acute hepatotoxicity in rats. Different groups of rats were administered 2 mg/kg bodyweight of paracetamol and monitored for morphological changes, biochemical parameters and histopathological changes. The result showed that the plant possesses hepatic protective potency in a dose dependent manner.

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals

All chemicals and reagents used were of analytical grades and products of reputable scientific and chemical companies such as British Drug House (BDH) chemical companies limited, green ford UK, Pool England. They include: Dimethyl sulfoxide (DMSO), methanol, distilled water, sodium hydroxide, chloroform, benzene, fehling's solution A and B, sulphuric acid, ammonium solution, ethylenediaminetetraacetic acid (EDTA), and normal saline. They were obtained from the Department of Biochemistry, Federal University of Technology, Minna, Nigeria.

2.2 Plant Collection

Fresh leaves of *Nelsonia campestris* were collected from the premises of Federal University of Technology, Minna, Niger State Nigeria in March 2013. Minna is located in the North-central region of Nigeria, with extreme weathers. *Nelsonia campestris* grows in the temperature range of 37-40°C. The plant sample was identified by a botanist at the herbarium section of Department of Biological Sciences, Federal University of Technology, Minna. The leaves were air dried at room temperature in the Department of Biochemistry Laboratory. The plant was pulverized into powder with pestle and mortar. The powdered sample was stored in a clean, labelled, polythene bag till ready for use.

2.3 Experimental Animals

Swiss albino rats of both sexes weighing between 200 and 250 g were procured from the Faculty of Pharmaceutical Sciences Ahmadu Bello University, Zaria, Nigeria. The animals were housed in plastic cages with wood shaving beddings. They were randomly allotted to five groups of five rats each, and allowed to acclimatize to laboratory condition for two weeks. They were allowed access to growers feed mesh and water *ad libitum* except for short fasting periods before oral administration of extracts.

2.4 Preparation of Plant Extract

Fifty gram (50 g) of powdered sample was extracted with 800 ml of distilled water by continuous refluxing for two hours at 100°C. The

extract was filtered with whatman no 2 filter paper, concentrated in water bath at 100°C, and stored in the refrigerator at -4°C till required for use. The percentage yield of the extract was noted.

2.5 Qualitative Phytochemical Screening

The aqueous extract of *Nelsonia campestris* was screened for the presence of phytoconstituents by the methods of [4,5].

2.6 Antimicrobial Activity Tests

Preliminary antibacterial analysis of the extract was carried out by agar diffusion method [6], while minimum inhibitory, and minimum bactericidal concentrations were determined by the method of [7].

2.7 Acute Toxicity Studies

The acute toxicity test of the plant extract was carried out by the method of [8]. Eighteen albino rats were grouped into six (each of three rats), and fasted overnight but allowed access to water. The rats were orally administered extract at varied doses of 100, 150, 250, 1000, 2000 and 5000 mg/kg bodyweights, and monitored for 24 hours to check for convulsion, salivation, diarrhoea, lethargy, sleep, coma, nervousness and/ or mortality.

2.8 Serum Enzyme Studies

2.8.1 Serum biochemical parameters

At the end of the experiment, the animals were fasted for 12 hours, anaesthetized under chloroform vapour, and sacrificed. Blood samples were collected by carotid puncture into heparinized tubes, centrifuged at 1000 rpm for 5 minutes and the clear serum supernatant was used freshly for the assessment of lipid profile, liver, and kidney function tests. For the haematological parameters, the blood was collected into EDTA test tubes.

2.8.2 Haematological parameters

Blood samples were collected into EDTA bottles. The full blood count includes; total red blood cell (RBC), white blood cell (WBC), and packed cell volume (PCV) were determined using Swelab Auto Haematology Analyzer.

2.8.3 Plasma lipid profiles

The plasma total cholesterol (TC), and triglyceride (TG), were determined using Randox diagnostic kits. The absorbance was calculated using Stat fax 4500 semi- automated chemistry analyzer.

2.8.4 Liver and kidney function tests

Serum enzymes: aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), total protein, creatinine, and urea were determined using Randox diagnostic kits.

2.9 Statistical Analysis

Statistical Product for Social Solution (SPSS) software was used to analyse all the data. Results are expressed as Mean \pm Standard error mean (SEM) using one-way analysis of variance (ANOVA), followed by Turkey, Duncan and Dunnet's multiple comparison test and ($p < 0.05$) were considered to be statistically significant.

3. RESULTS

Table 1 shows the qualitative phytoconstituents of aqueous extract of *N. campestris*, with high presence of flavonoids, steroids, anthraquinones, and saponins.

The bacterial susceptibility test for the extract is indicated in Table 2. At the dose of 360 mg/ml, *Staphylococcus aureus*, and *Salmonella typhi* showed the zones of inhibition greater than the standard drug.

Table 1. Qualitative phytochemical constituents of aqueous extract of *Nelsonia campestris*

Phytochemical	Aqueous extracts
Tannins	++
Flavonoids	+++
Phenols	++
Steroids	+++
Anthroquinone	+++
Terpenes	+
Alkaloids	-
Cardiac glycoside	++
Saponins	+++

Key- not detected, + mildly present, ++ moderately present, +++ highly present

Except for Pa, Bs, and Ec at 90 mg/ml, all bacterial pathogens recorded zones of inhibition in the range of (60-90) mg/ml.

Minimum bactericidal concentration is presented in Table 4 and is in the range of (60-90) mg/ml.

Single oral administration of the extract at doses of 10-5000 mg/kg bodyweight did not produce signs of toxicity, behavioural changes, and mortality in animals within 24 hours post administration.

4. DISCUSSION

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective and disease preventive properties (phytochemicals). These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others. The active principles of many drugs found in plants are these secondary metabolites [9]. Experimental screening method is imperative in order to establish the safety and efficacy of traditional and herbal products and to establish their active components [10].

The active components detected from the phytochemical screening of *Nelsonia campestris* extract (Table 1) are known to have curative properties against several pathogens [11]. It therefore justifies the traditional use of *N. campestris* in the treatment of gastrointestinal and respiratory complications associated with measles.

The bacterial susceptibility test of the extract recorded appreciable zone of inhibitions at doses of 320 and 360 mg/ml and compared favourably with the standard drug- amoxicillin (Table 2). This result agrees with that of the earlier researchers [12]. The extract inhibited only the growth of *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Escherichia coli* at various concentrations (Table 3). The reason may be that the chemotherapeutic agent (extract) acts on a specific target, and resistance arises when the target enzyme or organelle is modified so that it is no longer susceptible to the extract. In the like manner, many bacterial pathogens resist attack by inactivating drugs through chemical modification (as can be seen in the hydrolysis of the β -lactam ring of many penicillins by the enzyme penicillase). The extract also exhibited

minimum bactericidal effect on the pathogens as observed in various MBC values (Table 4). The result in Table 3 contrasts that of Table 4 as some pathogens in Table 3 that showed no zones of inhibition and recorded no growth during the MBC test seen in Table 4.

Acute oral toxicity test showed that the extract demonstrated high safety margin as the animals tolerated up to 5000 mg/kg bodyweight in both the two phases of the experiment (Table 5). The extract may therefore be assigned class 5 (LD50>5000), in the lowest toxicity class [13].

Table 2. Bacterial susceptibility test for aqueous extract of *Nelsonia campestris*

Concentration of extract (mg/ml)	Zone of inhibition of the pathogen(mm)						
	Pa	Sa	Bs	Kp	Sd	Ec	St
240	-	-	-	-	-	-	-
280	-	-	-	-	-	-	-
320	25	20	18	30	18	25	25
360	28	30	20	20	20	32	30
Standard drug	28	29	22	38	21	40	27

Key: Pa-*Pseudomonas aeruginosa*, Sa-*Staphylococcus aureus*, Bs-*Bacillus subtilis*, Kp-*Klebsiella pneumonia*, Sd-*Shigella dysenteriae*, Ec-*Escherichia coli*, St-*salmonella typhi*, (-)- No zone of inhibition, standard drug- amoxycillin (at 50 mg/ml)

Table 3. Minimum inhibitory concentration (MIC) of aqueous extract of *Nelsonia campestris*

Concentration of extract (mg/ml)	Pathogens						
	Pa	Sa	Bs	Kp	Sd	Ec	St
90	-	μ	-	μ	μ	-	-
80	μ		μ			μ	-
70	+	+	+	+	+	+	+
60	+	+	+	+	+	+	+
MIC	80	90	80	90	90	80	90

Key: Pa-*Pseudomonas aeruginosa*, Sa-*Staphylococcus aureus*, Bs-*Bacillus subtilis*, Kp- *Klebsiella pneumonia*, Sd-*Shigella dysenteriae*, Ec- *Escherichia coli*, St-*Salmonella typhi*, (-)=no turbidity observed, (+)= turbidity observed, μ= MIC value

Table 4. Minimum bactericidal concentration (MBC) of aqueous extract of *Nelsonia campestris*

Concentration of extract	Pathogens						
	Pa	Sa	Bs	Kp	Sd	Ec	St
60	+	+	+	+	+	+	+
70	+	+	+	+	+	+	+
80	+	+	π	+	+	Π	+
90	+	π	+	π	π	+	Π
MBC	Nil	90	80	90	90	80	90

Key: Pa-*Pseudomonas aeruginosa*, Sa-*Staphylococcus aureus*, Bs-*Bacillus subtilis*, Kp-*Klebsiella pneumonia*, Sd-*Shigella dysenteriae*, Ec-*Escherichia coli*, St-*salmonella typhi*, (-)= growth observed, (+)= no growth observed, π= MBC value

Table 5. Acute oral toxicity test of extract (Ethyl acetate) leaf extract of *Nelsonia campestris*

Phase 1		Phase 2	
Dose (mg/kgbw)	Mortality	Dose (mg/kgbw)	Mortality
10	0/3	2000	0/3
100	0/3	3000	0/3
1000	0/3	5000	0/3

Anaemia resulting from excessive intake of herbal preparation may be due to haemolysis of red blood cells or inhibition of blood cell synthesis by active constituents of the extract. The extract at all doses showed no significant differences ($p < 0.005$) in the levels of RBC, and PCV when compared with the control group. However, at high dose of 5000 mg/kg bodyweight of extract, there was a non-significant ($p > 0.05$) decrease in PCV level. There was no significant difference ($p < 0.05$) in WBC among the treated groups but there was an increase in WBC compared to that of the control groups (Fig. 1). This increase in WBC was likely triggered by the metabolic assault from phenolic contents or other phytochemicals in *N. campestris*. This finding agrees with the work of [14] that phytochemicals could trigger the release of white blood cell in the body. The active constituents of *N. campestris* therefore did not cause lyses of blood cells or inhibition in blood cells synthesis, since there was no reduction in haematological parameters.

The decrease in protein was common among all groups when compared to the control group (Fig. 2). This may be the result of decreased deamination or decreased synthesis of protein by the liver. The decreased in creatinine may be due to low glomeruli filtration, because it is usually not reabsorbed. Elevation in urea in the treated groups correlates with the earlier study by 12 that reported a significant increase in urea when administered high doses of extract of *Sclerocarya birrea*. This elevation may probably be due to large amount of ammonia formed.

Administration of the aqueous extract exhibited a reduction in cholesterol among treated groups

compare to the control group (Fig. 3). This shows that the extract may possess active components that competitively inhibit hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, a rate limiting enzyme in the synthesis of cholesterol. This inhibition decreases cholesterol synthesis. The lowering activity has some beneficial effect on cardiovascular risk factors [15]. The extract (Table 1) had high presence of flavonoids which may be responsible for the low cholesterol recorded (Fig. 3). Several research conducted had indicated that many plant sterol reduce cholesterol absorption [16], and according to [17,18], flavonoids in extracts provide lipid lowering effect.

The measurement of activities of enzymes in tissue and body fluid plays a significant in disease investigation and diagnosis [19]. Tissue enzymes assay can also indicate tissue cellular damage long before structural damage can be picked by conventional histological techniques. Such measurement can give an insight to the site of cellular tissue damage as a result of assault by the plant extract. Liver cell damage is characterized by a rise in plasma enzymes (aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT)). Serum AST activity increased in all groups in a dose dependent fashion in reference to the control (Fig 4). Highest activity was observed at 2000 mg/kg bodyweight. A rise in AST level in the serum could not rule out the possibility of hepatocellular damage or myocardial ischemia caused by administration of the extract.

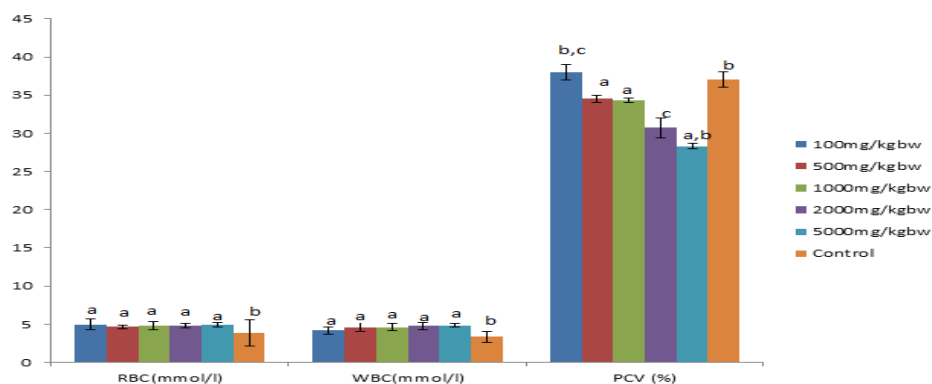


Fig. 1. Effect of aqueous extract of *Nelsonia campestris* on some haematological parameters
 The PCV values of animals in all groups reduced significantly ($p > 0.05$) except the dose of 100 mg/kg bodyweight. RBC, and WBC however increased significantly ($p < 0.05$) in reference to the control group

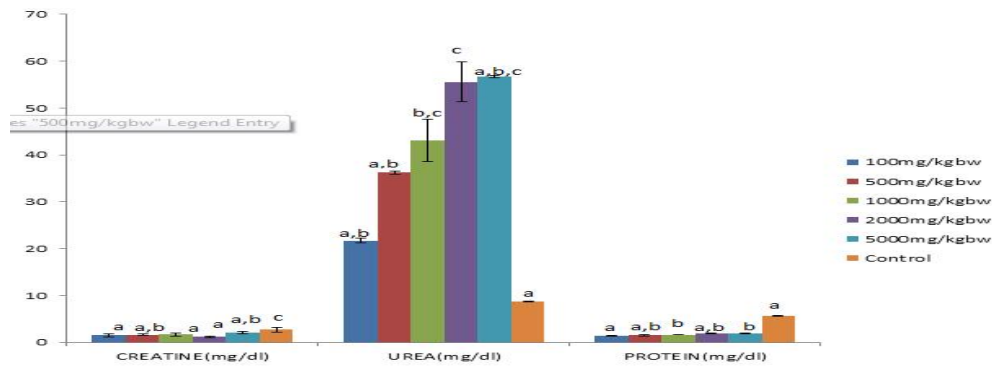


Fig. 2. Effect of aqueous extract of *Nelsonia campestris* on creatinine, urea and total protein
Creatinine, total protein, and urea increased significantly ($p < 0.05$) in all groups in reference to the control group

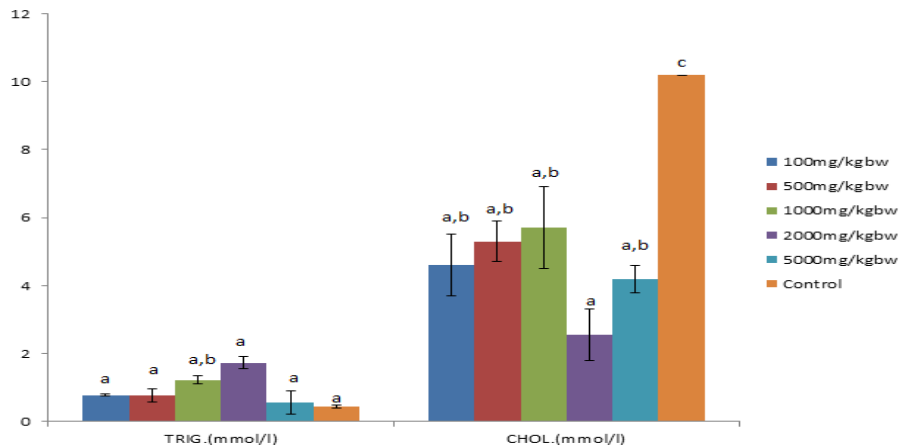


Fig. 3. Effect of aqueous extract of *Nelsonia campestris* on cholesterol and triglyceride
Cholesterol concentration decreased ($P > 0.05$) significantly in all the treated groups while that of triglyceride increased significantly ($p < 0.05$)

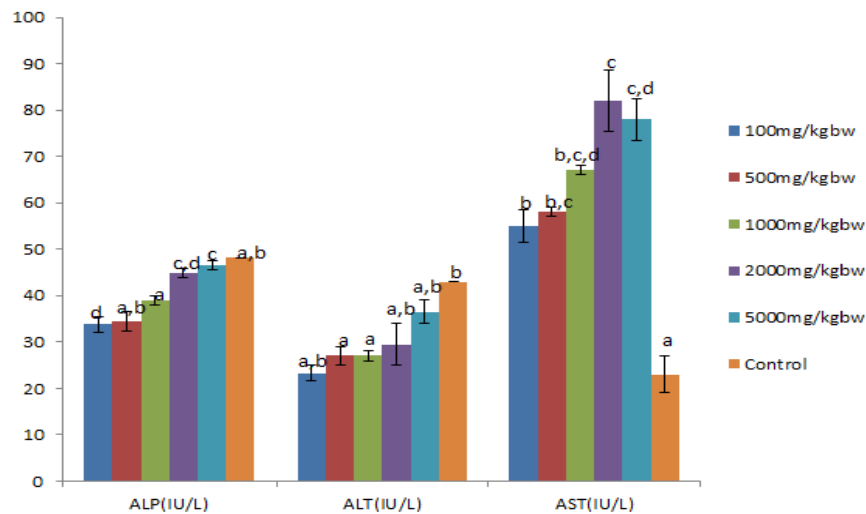


Fig. 4. Effect of aqueous extract of *Nelsonia campestris* on some liver enzymes
Activity of AST increased significantly ($p < 0.05$) while that of ALP and ALT decreased significantly ($p > 0.05$)

5. CONCLUSION

The aqueous extract of *Nelsonia campestris* was able to experimentally inhibit the growth of bacterial pathogens as claimed by its folkloric use. The extract was also able to reduce cholesterol compared to the control group indicating that the plant may provide preventive measure for cardiovascular disease. The extract may also cause mild organ toxicity as seen in the activities of liver enzymes. This preliminary study therefore provides the basis for further study on detailed toxic and pharmacological effects of the aqueous extract of *N. campestris* and a possible discovery of a novel drug from the plant.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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