

Original Research Article

INVITRO ANTIBACTERIAL ACTIVITY AND INVIVO ACUTE TOXICOLOGICAL STUDIES OF *NELSONIA CAMPESTRIS* AQUEOUS LEAF EXTRACT

Abstract

Invitro antibacterial activity and *invivo* toxicological studies of aqueous extract of *Nelsoniacampestris* was investigated using standard laboratory procedures. The extract was highly active against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Klebsiella pneumonia* and moderately active against *Bacillus subtilis* and *Shigelladysenteriae* with inhibition diameters in the range of (20mm-30mm). 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. 100mg/kg, 500mg/kg, 1000mg/kg, 2000mg/kg and 500mg/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 groups. 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. Aqueous extract of *Nelsoniacampestris* has activity against the microbes resulting from the suppression of immunity by morbillivirus, and hypolipidemic effects.

Keywords: Antibacterial, Hypolipidemic, Haematological, Biochemical, Immunity

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. *Nelsoniacampestris* 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 (Piemeet *al.*, 2006). 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 *Nelsoniacampestris* belonging to the family of Acanthaceae, and is a useful plant of tropical Africa, and Australia. 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 *Nelsoniacampestris* 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 *et al.*, 2012 *Nelsoniacampestris* 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 2mg/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.

2.0 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: Dimethylsulfoxide (DMSO), methanol, distilled water, sodium hydroxide, choloform, benzene, fehling's solution A and B, sulphuric acid, ammonium solution and normal saline. They were obtained from the Department of Biochemistry, Federal University of Technology, Minna, Nigeria.

2.2 Plant Collection

Fresh leaves of *Nelsoniacampestris* were collected from the premises of Federal University of Technology, Minna, Niger State, Nigeria in March 2013. 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 250g 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 (50g) 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 filter paper, condensed 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 crude aqueous extract of *Nelsoniacampestris* was screened for the presence of phyto-constituents by the methods of Trease and Evans (1989) and Sofowara (1993).

2.6 Antimicrobial Activity Tests

Preliminary antibacterial analysis of the extract was carried out by agar diffusion method (Sharma *et al.*, 1998), while minimum inhibitory, and minimum bactericidal concentrations were determined by the method of Pushpalatha *et al.*, 2010.

2.7 Acute Toxicity Studies

The acute toxicity test of the plant extracts were carried out by the method of (Lorke, 1983). 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.

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112 2.8.2 Hematological Parameters

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

116

117 2.8.3 Plasma Lipid Profiles

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

121 2.8.4 Liver and Kidney Function Tests

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

125 2.8.5 Statistical Analysis

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

130 3.0 RESULTS

131 Table 1: Qualitative phytochemical Constituents of Aqueous Extract of *Nelsoniacampestris*

Phytochemical	Aqueous Extracts
Tannins	++
Flavonoids	+++
Phenols	++
Steroids	+++
Anthroquinone	+++

Terpenes	+
Alkaloids	-
Cardiac glycoside	++
Saponins	+++

Key

- Not detected

+ Mildly present

++ Moderately present

+++ Highly present

Table 2: Bacterial Susceptibility Test for Aqueous Extract of *Nelsoniacampestris*

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	30	20	32	30
Standard Drug	2829	22	38	21		40	27

KEY:

Pa-*Pseudomonasaeruginosa*

Sa-*Staphylococcus aureus*

Bs-*Bacillus subtilis*

Kp-*Klebsiella pneumonia*

Sd-*Shigelladysenteriae*

155 *Ec-Escherichia coli*
 156 *St-Salmonella typhi*
 157 (-)- No zone of inhibition
 158 Standard drug- Amoxycillin (at 50 mg/ml)
 159
 160

161 **Table 3:** Minimum Inhibitory Concentration (MIC) of Aqueous Extract of *Nelsoniacampestris*

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

171 KEY:
 172 Pa-*Pseudomonasaeruginosa*
 173 Sa-*Staphylococcus aureus*
 174 Bs-*Bacillus subtilis*
 175 Kp-*Klebsiella pneumonia*
 176 Sd-*Shigelladysenteriae*
 177 Ec-*Escherichia coli*
 178 St-*Salmonella typhi*
 179 (-)=No Turbidity observed
 180 (+)= Turbidity observed
 181 μ= MIC value
 182

Table 4: Minimum Bactericidal Concentration (MBC) of Aqueous Extract of *Nelsoniacampestris*

Concentration of extract (mg/ml)	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-*Pseudomonasaeruginosa*
 Sa-*Staphylococcus aureus*
 Bs-*Bacillus subtilis*
 Kp-*Klebsiella pneumonia*
 Sd-*Shigelladysenteriae*
 Ec-*Escherichia coli*
 St-*Salmonella typhi*
 (-)= Growth observed
 (+)= No growth observed
 π= MBC value

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Extract	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

Table 5: Acute Oral Toxicity Test of Leaf Extract of *Nelsoniacampestris*

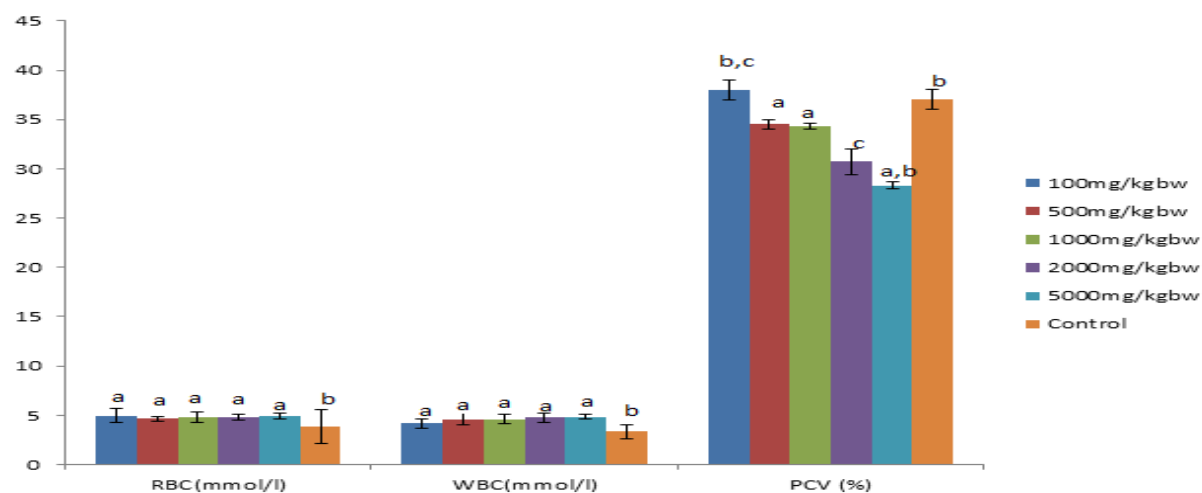


Figure 1: Effect of Aqueous Extract of *Nelsoniacampestris* on some Hematological parameters

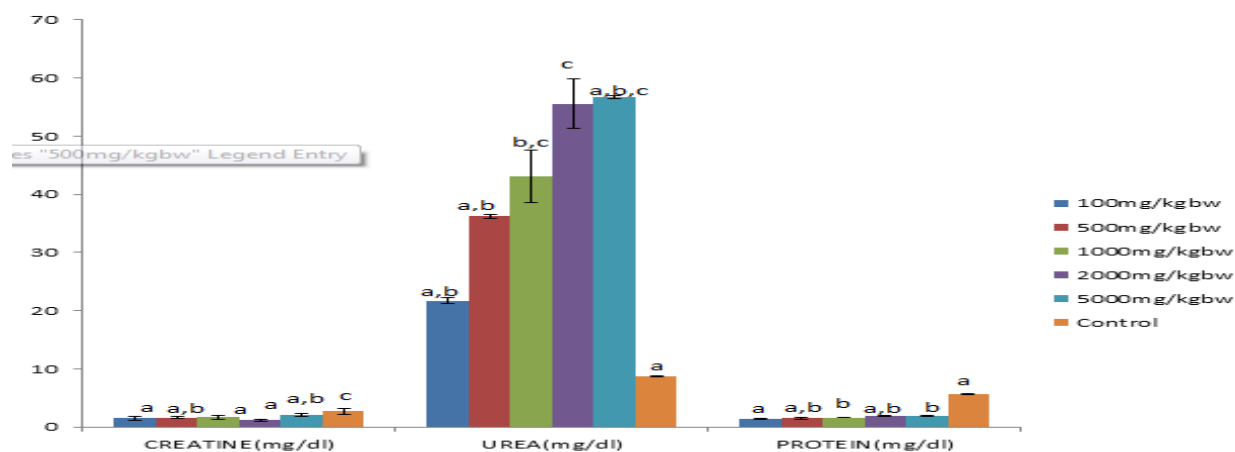


Figure 2: Effect of Aqueous Extract of *Nelsoniacampestris* on Creatinine, Urea and Total protein

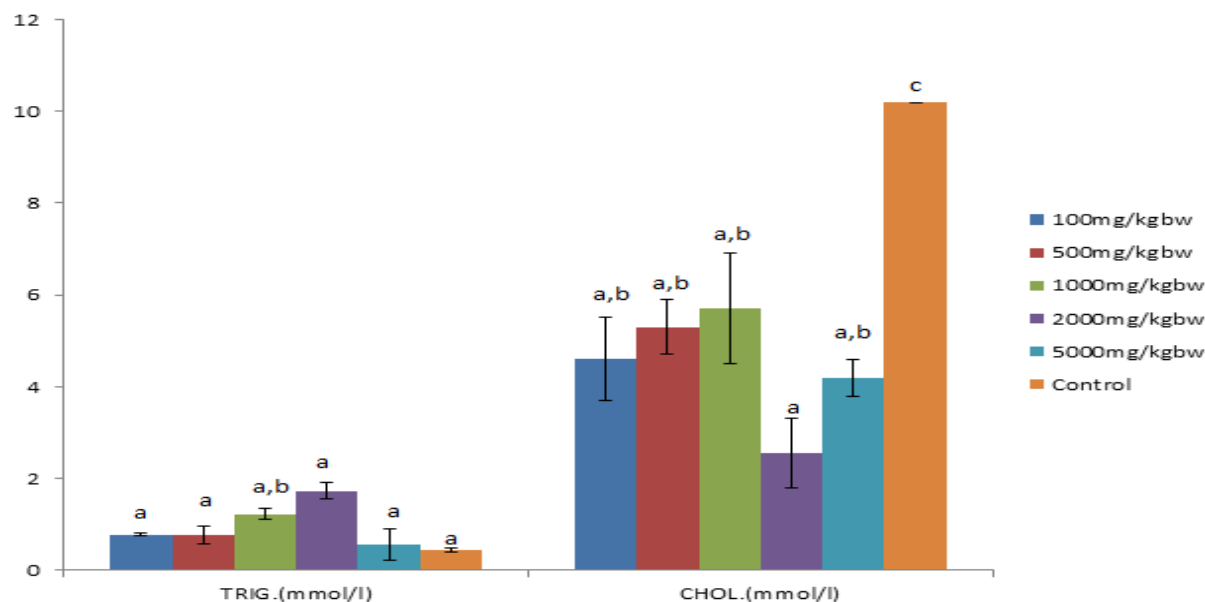


Figure 3: Effect of Aqueous Extract of *Nelsoniacampestris* on Cholesterol and Triglyceride

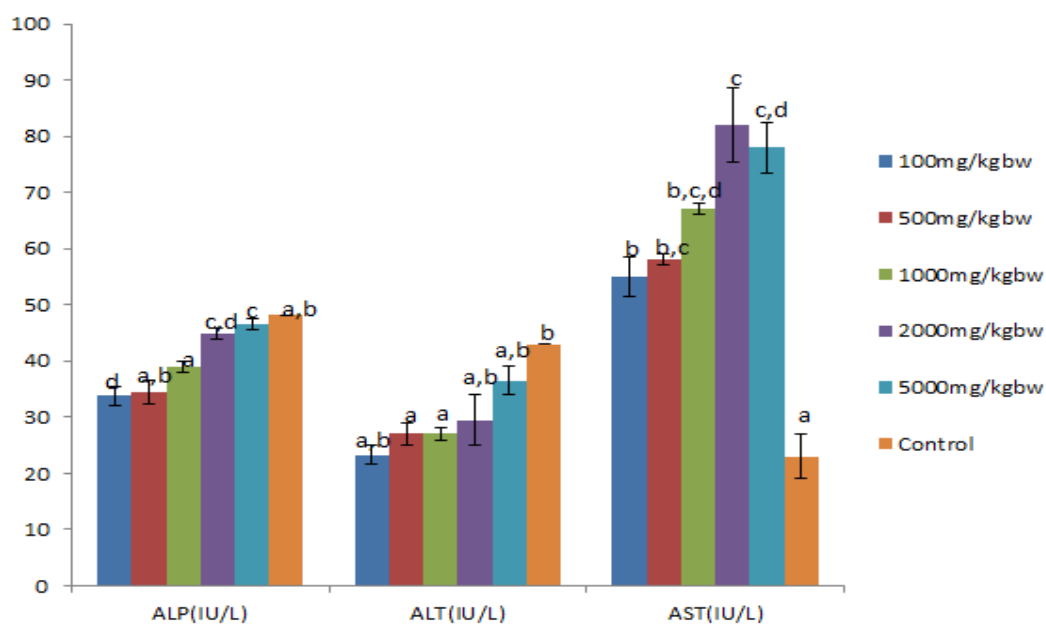


Figure 4:Effect of Aqueous Extract of *Nelsoniacampestris* on some Liver Enzymes

4.0 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 (Ghani, 1990). Experimental screening method is imperative in order to establish the safety and efficacy of traditional and herbal products and to establish their active components (Mythilypriyaet *al.*, 2007).

The active components detected from the phytochemical screening of *Nelsoniacampestris* extract (Table 1) are known to have curative properties against several pathogens (Owoyele *et al.*, 2005). 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 (Esonuet *et al.*, 2001). 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 ($LD_{50} > 5000$), in the lowest toxicity class (OECD, 2001).

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 (Figure 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 Ostlund *et al.*, 2003 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 (Figure 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 Muhammad *et al.*, 2011 that reported a significant increase in urea when administered high doses of extract of *Sclerocaryabirrea*. This elevation may probably be due to large amount of ammonia formed.

The aqueous extract exhibited a reduction in cholesterol among treated groups compared to the control group (Figure 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 (Dixit *et*

al., 1992). According to Zhou *et al.*, 2009; Sharma *et al.*, 2008; and Viana *et al.*, 2004, flavonoids in extracts provide lipid lowering effects. The aqueous extract of *Nelsoniacampestris* (Table 1) had high presence of flavonoids which may be responsible for the low cholesterol recorded (Figure 3). Several research conducted had indicated that many plant sterol reduce cholesterol absorption (Sushruta *et al.*, 2006).

The measurement of activities of enzymes in tissue and body fluid plays a significant in disease investigation and diagnosis (Malomo, 2000). 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. 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.

CONCLUSION

The low level of triglyceride and cholesterol compared to the control group indicates the plant may provide a preventive measure for cardiovascular disease. This preliminary study provides the basis for further study on detailed toxic and pharmacological effects of the extract of *N. campestris* and a possible discovery of a novel drug from the plant.

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REFERENCE

- 310 Asanga, E., Edet, E. E. P., and Eseyin, A. O. (2014). Hematological parameters of alloxan-induced
311 diabetic rats treated with ethanol extracts and fractions of *nauclea lafilioia* leaf. *European*
312 *Scientific Journal*. **9**(27): 203-210.
- 313 Gupta B., Kalita J., Chowonvry A., and Kotoky J (2012). Hepatoprotective activity of
314 *Nelsoniacampestrison* acute hepatotoxicity induced by paracetamol. *International journal*
315 *of pharmacy and pharmaceutical science* **4** (3):107-120
- 316 Dixit, V.P., Varma, M., Marthur, N.T., Marthur, R., and Sharma, S. (1992).
317 Hypocholesterolaemic and Anti-arteriosclerotic effects of Solasodine (C₂₇H₄₂O₂N) in
318 cholesterol-Fed rabbits. *Phytother. Res.*, **6**, 270-273.
- 319 Esonu, B.O., Emenalom, O.O., Udedibie, A. B. I., Herbert, U., Ekpor, C. F., Okoli, I. C and
320 Ihukwumere F.C. (2001). Performance and blood chemistry of weaner pigs fed raw
321 *Mucuna* beans (Velvet bean) meal. *Tropical Animal Production Investment*. **4**: 49-54.
- 322 Ghani, A. (1990). Introduction to Pharmacognosy. Ahmadu Bello University Press, Zaria, Nigeria.
- 323 Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*. **54**:
324 275-287.
- 325 .Malomo, S.O. (2000). Toxicological Implication of Ceftriaxone Administration in Rats. *Nigerian*
326 *Journal of Biochemistry and Molecular Biology*. **15**(1): 33-38.
- 327 Muhammad, S., Hassan, G., Dangoggo, S.M., Hassan, S.W., Umar, K.J and Aliyu R.U (2011).
328 Acute and subchronic toxicity studies of kernel extract of *Sclerocarya birrea* in rats.
329 *Science World Journal* **6**(3): 70-75
- 330 Mythilypriya, R., Shanthi, P., and Sachdanandam, P. (2007). Oral acute and Sub acute toxicity
331 studies of *Kalpamrutha*, a modified indigenous preparation on rats. *Journal of Health*
332 *Sciences*. **53** (4):351-358.
- 333 Organization for Economic Cooperation and Development (OECD) (2001). OECD guidance
334 document on oral toxicity testing. Organization for Economic Cooperation and
335 Development. Paris France.
- 336 Ostlund, R.E., Recette, S.B. and Stenson, W. (2003). Inhibition of Cholesterol Absorption by
337 Phytosterol-Replete Wheat Germ Compared with Phytosterol-Depleted Wheat Germ.
338 *American Journal of Clinical Nutrition*. **77** (6): 1385.
- 339 Owoyele ,V.B., Oloriegbe, Y.Y. Balogun, E.A .and Soladoye A.O (2005). Analgesic and anti-
340 inflammatory properties of *Nelsoniacanescence* leaf extract. *Journal of*
341 *Ethnopharmacology* **99**(1) 153-6.

- 342 Pieme, C.A., Penlap, V.N., Nkegoum, B., Taziebou, C.L., and Ngongang, J (2006). Evaluation of
343 acute and subchronic toxicities of aqueous and methanolic extracts of leaves of
344 *Senna alata* (L.) Roxb (Cesalpiniaceae). *African Journal of Biotechnology*. 5(1): 283-
345 289.
- 346 Pushpalatha, B., Rama, M., Reddy, L., Mannur, I.S., and Vijaya, T (2010). Medicinal plants and
347 their derivatives as potential source in the treatment of obesity. *Asian Journal*
348 *of Experimental Biological Science*. 1(14): 719-727
- 349 Sharma, B., Balomajumder, C., and Roy, P. (2008). Hypoglycemic and Hypolipidemic effects of
350 Flavonoid rich extract from *Eugenia Jambolana* seeds on Streptozotocin induced Diabetic
351 rats. *Food and Chemical Toxicology*. 7. 2376- 2383.
- 352 Sofowora, A. 1993. Medicinal Plants and Traditional Medicines in Africa. Chichester John Wiley
353 & Sons
354 New York, pp: 97-145
- 355 Sushruta, K., Satyanarayana, S., Srinivas, N., Sekhar, S. and Raja, J. (2006). Evaluation of the
356 Blood-Glucose Reducing Effects of Aqueous Extracts of the Selected Umbelliferous
357 Fruits Used in Culinary Practice. *Tropical Journal of Pharmaceutical Research*. 5: 613-
358 617.
- 359 Trease, G.E. and W.C. Evans, 1989. Pharmacology 11th Edn., Bailliere Tindall Ltd., London, pp:
360 60-75.
- 361
362 Viana, S.B., Medeiros, C.C., Lacerda, A.C., Leal, A.M., Vale, T.G. and Matos, F.A.
363 (2004). Hypoglycemic and Anti-Lipemic Effects of the Aqueous Extract from
364 *Cissus Sicyoides*. *Biomedical Central Pharmacology*. 4:9.
- 365
366 Zhou, T., Lou, D., Li, X., and Luo, Y. (2009). Hypoglycemic and hypolipidemic effects of
367 Flavonoids from lotus (*Nelumbo nucifera* Gaertn) leaf in Diabetic mice. *Journal of*
368 *Medical Plant Research*. 3, 290-293.
- 369
370