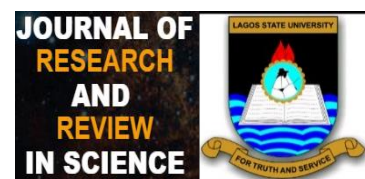


ORIGINAL RESEARCH

Markers of Liver function in rabbits (*Oryctolagus cuniculus*): Bark extracts of *Nauclea latifolia* and *Alchornea cordifolia* plants

Olabisi Ogunrinola*, Olusegun Fajana, Samuel Olaitan, Oluwatosin Adu, Millicent Nuno, Babajide Elemo

Department of Biochemistry, Faculty of Science, Lagos State University, Ojo, Lagos, Nigeria



Abstract:

Introduction: The use of *Nauclea latifolia* and *Alchornea cordifolia* plants as herbal curative medicine is fast growing and the need to investigate the likely toxicity to avert severe medical issues.

Aims: To assess the toxicity level, the liver function enzyme activities in rabbits (*Oryctolagus cuniculus*) administered with both ethanolic bark extracts of *Nauclea latifolia* and *Alchornea cordifolia* were studied.

Materials and Methods: Eighty-four (84) rabbits were arbitrarily grouped into seven (n = 12), where group 1 is the control, and the remaining groups were orally administered with 500, 750 and 1000 mg/kg body weight of ethanolic bark extract of *Nauclea latifolia* and *Alchornea cordifolia* (LD50>1000mg/kg). In each group, after 24 hours of administration on the 1st, 3rd and 7th days, three animals were sacrificed. Markers of liver functions: serum alkaline phosphate (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes activities were monitored using spectrophotometry instrument.

Results: The results show a significant (p < 0.05) reduction of ALP, AST and ALT activities at all doses for the days of administration of both ethanolic bark extracts of *Nauclea latifolia* and *Alchornea cordifolia* when compared to the control.

Conclusion: The study suggests that ethanolic bark extracts of *Nauclea latifolia* and *Alchornea cordifolia* may possess relative hepatoprotective activity.

Keywords: *Nauclea latifolia*, *Alchornea cordifolia*, Herbal, rabbit, alkaline phosphate, aspartate aminotransferase, alanine aminotransferase

Correspondence

Olabisi Ogunrinola, Department of Biochemistry, Faculty of Science, Lagos State University, Ojo, Lagos, Nigeria.
Email:olabisi.ogunrinola@lasu.edu.ng; ooogunrinola@yahoo.com

All co-authors agreed to have their names listed as authors.

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1. INTRODUCTION

The center of metabolic homeostasis is the liver where detoxification and excretion of many xenobiotic compounds occurs. Liver metabolic dysfunctions are linked to up or/down serum concentrations of many biological markers like transaminases and phosphatases [1-3]. For centuries, traditionalist uses medicinal plants known to contain components that are precursors for the synthesis of drugs as remedies to for disorder, illness and diseases in human. These plant components can either destroy or prevent the development of pathogens [4-6].

The use of indigenous medicinal plants plays an essential role in the management and treatment of a variety of diseases [7] and to cure symptoms of various parasitic, fungal and bacteria illness and diseases [8-15]. *Nauclea latifolia* (Rubiaceae) plant is found in the forest and fringe tropical forest. Different parts (leaves, root, and stem) of *N. latifolia* is used by traditional specialists in the treatment of ailments like malaria, gastrointestinal tract disorders, sleeping sickness, prolong menstrual flow, and hypertension [16]. The presence of biological active principles such as alkaloids, terpenes, saponins, and polyphenols, justifies its reported metabolic dysfunction management [17]. *Alchornea cordifolia* (*A. cordifolia*) plant belong to the Family Euphorbiaceae. It is traditionally used in Nigeria as topical anti-inflammatory, antioxidant, antidiarrhoeal, antibacterial and antifungal drugs [18-20] due to the active phytochemical constituents from the leaves, roots and stems. Some plant metabolites can have a toxic effect on the host liver during the biotransformation stage that result of cell damage [21], and needs to evaluate the quality, efficacy and standard of plant drug formulations as safer drugs in order to assess toxicity risks associated with usage. In view of this, the present study report the efficacy effect of *Nauclea latifolia* and *Alchornea cordifolia* on markers of liver functions in rabbits.

2. MATERIAL AND METHODS

2.1 Plant collection and extract preparation

The air-dried bark of *Nauclea latifolia* and *Alchornea cordifolia* plants were obtained from the Oyingbo market, Lagos Mainland, Nigeria, identified in the Department of Botany, Lagos State University, Ojo, Nigeria. 300 g dried bark of both *Nauclea latifolia* and *Alchornea cordifolia* were chopped into pieces and extracted with 70% ethanol for 4 hours using soxhlet apparatus. The collected crude ethanolic bark extracts of *Nauclea latifolia* and *Alchornea cordifolia* was stored for further usage.

2.2 Experimental animals and protocol

84 male rabbits (80 - 140g) were kept under 12/12 h light / dark cycle and free access to food (Ladokun and Sons Livestock Feeds Ltd, Nigeria) and water for 14 days preceding the experiment. The animals were informally divided into seven groups (n = 12). Group one is the control and groups' two to seven were administered orally with 500, 750 and 1000mg/kg body weight of crude ethanolic extracts of *Nauclea latifolia* and *Alchornea cordifolia* bark for seven days. The animals were sacrificed under light anesthesia at the end of 1st, 3rd, 5th, 7th days respectively. Blood was collected and centrifuged (Biocotek Model 90-1) at 4000 rpm for 15 minutes at 4°C to separate the serum for biochemical analyses. The investigational protocols are performed in alliance with the principles for laboratory animal use and care [22], which was approved by the Animal Ethical Committee, Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria.

2.3 Determination of serum biochemical parameters

Activities of serum alkaline phosphatase (ALP) [23], aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [24], were examined with commercial diagnostic kits (Randox Laboratories Ltd, UK) using colorimetric instrument of Surgienfield Instrument, Zhejiang, China, Mainland.

2.4 Statistical analysis

All statistical analyses were done with IBM SPSS (version 17.0) software (IBM Corp., Armonk, NY, USA). And data were expressed as Mean±SEM of three replicates. The analysis of ANOVA for the level of homogeneity at $p < 0.05$ among the groups was performed.

3. RESULTS

Figure 1 depicted the result of serum Alkaline phosphatase (ALP) activity in animal administered with ethanolic bark extracts of *N. latifolia* (Fig. 1a) and *A. cordifolia* (Fig. 1b). The study revealed a significant ($p < 0.05$) reduction in serum activity of ALP on all the days of administration with ethanolic bark extracts of *N. latifolia* and *A. cordifolia* at all doses compared to the control. In both the extracts, up/down regulation of the ALP activity was observed among the dosage groups.

The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in animal administered with bark *N. latifolia* and *A. cordifolia* ethanolic extracts are revealed in figure 2 and 3 respectively. Fig. 2a shows that at different doses of *N. latifolia*, there was a significant ($p < 0.05$) decreased in the serum AST activity for the seven days compared to

the control. Although, treatment with 500 mg/L has the least AST activity compared to the 1000 mg/L group. A similar result was observed with the administration of *A. cordifolia* (Fig. 2b).

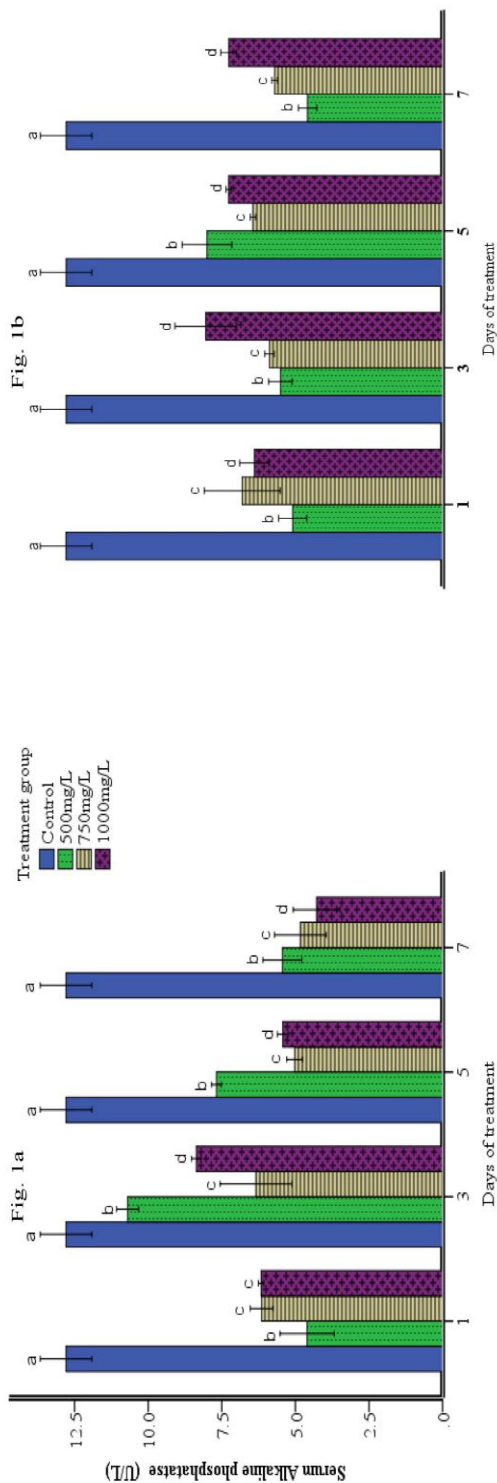


Fig. 1. Serum alkaline phosphatase activity (ALP) following daily administration of ethanolic extract of *Nauclea latifolia* (Fig. 1a) and *Alchornea cordifolia* (Fig. 1b) for seven days. Each bar represents the mean values \pm Standard error of means of three rats. Bars with different alphabets are significantly different at $p < 0.05$.

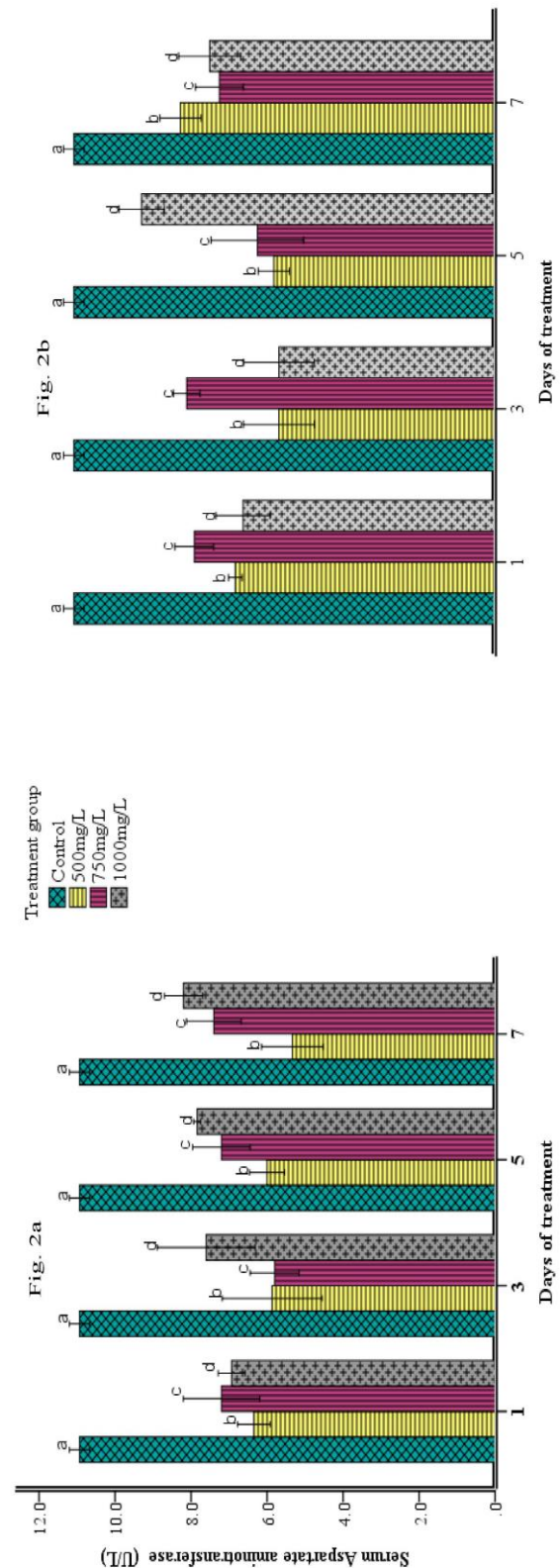


Fig. 2. Serum aspartate aminotransferase (AST) activity following daily administration of ethanolic extract of *Nauclea latifolia* (Fig. 2a) and *Alchornea cordifolia* (Fig. 2b) for seven days. Each bar represents the mean values \pm Standard Error of Means of three rats. Bars with different alphabets are significantly different at $p < 0.05$.

In Fig. 3, a significant ($p < 0.05$) reduction of ALT activity with the administration of different doses of ethanolic extracts of *N. latifolia* and *A. cordifolia* was

detected. The observed reduction of serum ALT activity with *N. latifolia* (Fig. 3a) at different doses is lower than that of *A. cordifolia* (Fig. 3b) at the end of day seven.

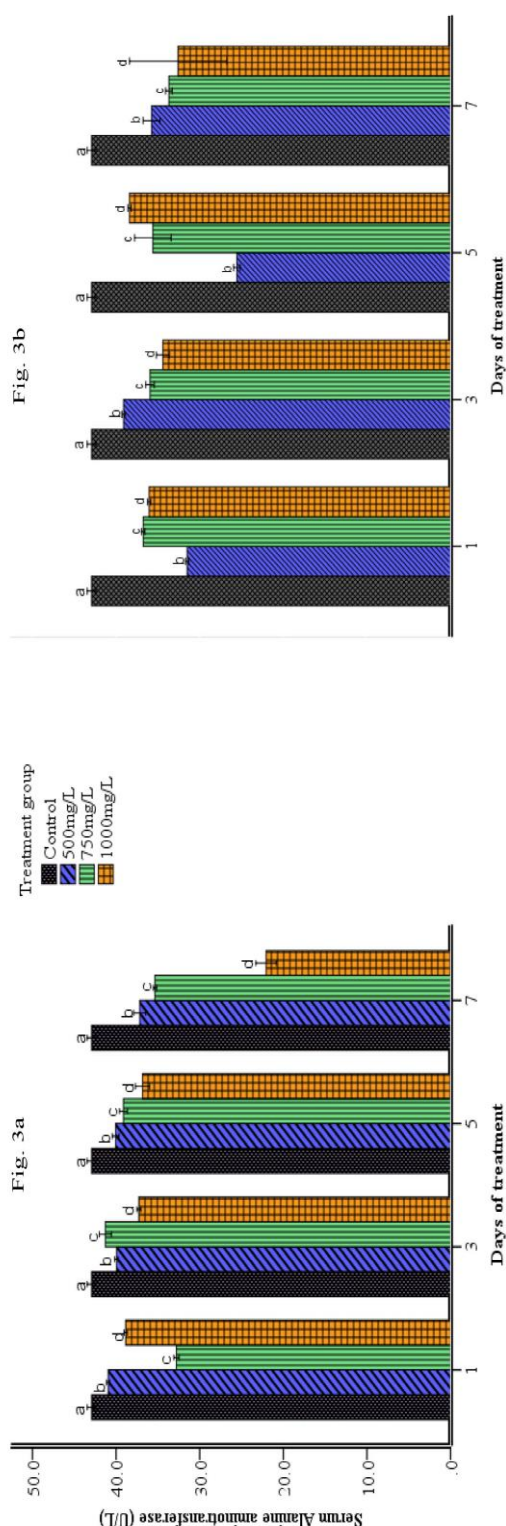


Fig. 3. Serum alanine aminotransferase (ALT) activity following daily administration of ethanolic extract of *Nauclea latifolia* (Fig. 3a) and *Alchornea cordifolia* (Fig. 3b) for seven days. Each bar represents the mean values \pm Standard Error of Means of three rats. Bars with different alphabets are significantly different at $p < 0.05$.

The serum ratio of aspartate aminotransferase (AST) to alanine aminotransferase (ALT) activities in animal administered with *N. latifolia* and *A. cordifolia* ethanolic bark extracts are represented in Table 1. At end of the seventh day, the AST: ALT ratio decrease for *N. latifolia* at 750 mg/L, increase at 500 mg/L and 1000

mg/L compared to control while a similar result was observed with the administration of *A. cordifolia* extract except that no AST:ALT ratio changes at dose of 750 mg/L.

Table 1. Ratio of aspartate aminotransferase (AST) to alanine aminotransferase (ALT)

Treatment group/Days	Days of treatment							
	1		3		5		7	
	<i>N. latifolia</i>	<i>A. cordifolia</i>	<i>N. latifolia</i>	<i>A. cordifolia</i>	<i>N. latifolia</i>	<i>A. cordifolia</i>	<i>N. latifolia</i>	<i>A. cordifolia</i>
Control	0.26 ± 0.01^a	0.26 ± 0.01^a	0.26 ± 0.01^a	0.26 ± 0.01^a	0.26 ± 0.01^a	0.26 ± 0.01^a	0.25 ± 0.01^a	0.25 ± 0.01^a
500mg/L	1.36 ± 0.01^b	1.98 ± 0.02^b	0.33 ± 0.01^b	0.32 ± 0.01^b	0.84 ± 0.02^b	0.88 ± 0.03^b	0.50 ± 0.04^b	1.29 ± 0.08^b
750mg/L	1.89 ± 0.06^c	1.87 ± 0.06^c	0.91 ± 0.04^c	0.76 ± 0.01^c	0.82 ± 0.01^b	0.09 ± 0.01^c	0.86 ± 0.03^c	0.09 ± 0.01^c
1000mg/L	1.55 ± 0.04^d	2.07 ± 0.02^d	0.57 ± 0.01^d	0.98 ± 0.03^d	0.15 ± 0.01^c	0.25 ± 0.01^a	0.88 ± 0.06^c	1.16 ± 0.05^d

Superscripted items indicate significant values ($p < 0.05$) from control ($n = 3$). Results expressed as Mean \pm S.E.M = Mean Values \pm Standard error of mean of two experiments.

4. DISCUSSION

The diagnostic enzymes, ALP, AST and ALT activities are used in detecting specific hepatic biochemical changes in relation to diseases. ALP is known to be localized in mucosal epithelia of small intestine, proximal convoluted tubule of kidney, bone, liver and placenta to implement lipid transportation in the intestine and calcification in bone. Its concentration in the serum is mainly from the liver with 50% contribution from the bone [25]. Serum ALP level is a pointer of either liver function or bone metabolism due

to its participation in the transport of metabolites across the cell membranes, protein synthesis, secretory activities, and glycogen metabolism [26-28]. The observed significant decrease in serum activity of ALP on all the days of administration of ethanolic bark extracts of *N. latifolia* and *A. cordifolia* at all doses compared to the control is in accordance with the researches of Udobi *et al.* [29] and Olaleye *et al.* [30]. The reduction could be due to inhibiting or inactivating ALP in situ, as observed by Oloyede *et al.* [31] with Asa water and Akanji *et al.* [32] with metabisulphate, revealing healthy liver function.

AST is responsible for the metabolism through transamination of aspartate and used as a sensitive indicator of liver disease [33, 34]. The observed lowered serum AST activity of animals administered with ethanolic bark extracts of *A. cordifolia* is in agreement with the research of Udobi *et al.* [29], Olaleye *et al.* [30] and Akpanabiatu *et al.* [34].

Cytoplasmic enzyme ALT is localized in hepatocytes and it is released into the blood during the cell damage. The result of this study shows a significant dose dependent decrease in ALT activity with the administration of *N. latifolia* and *A. cordifolia* for seven days respectively. This decrease is in line with the reports of Osadebe *et al.* [19]; Akpanabiatu *et al.* [34]. The reduction may be the mechanism of action of both extracts in preventing leakage of intracellular enzymes by their membrane stability activities, thus improving the secretory mechanism of the hepatic cells. Report of Okuda *et al.* [35] suggested that tannins in medicinal plants are effective against liver injury by preventing the formation of lipid peroxide, thereby reducing effect in co-existing substances or preventing their oxidation. Therefore, the presence of tannin or other active components in the plants could be the extract's protective effects.

The ratio of serum AST to ALT helps to identify the etiology of any underlying liver dysfunction. The study observed high ratio with the high dose on the seventh day, which is in accordance with the report of Shreevastva *et al.* [36]. This rise in ratio observed in this study may be due to the ethanol as a solvent of the plant extract. It also reflects either there was a relative increase in AST activity or a relative decrease in ALT activity by the administration of ethanolic bark extracts of *N. latifolia* and *A. cordifolia* at highest dose. These changes may have been due to relative changes in their release, synthesis, or metabolic distribution and clearance in the liver, although the liver AST and ALT was not investigated.

4. CONCLUSION

Derivatives of medicinal plant are used in folk medicine because they consist of molecules with pharmacological properties required for life sustainability for protecting or preventing illness and

diseases thus, the safety of the plants are of great concern. Studies have revealed that ethanolic bark extracts of both *N. latifolia* and *A. cordifolia* consist of phytonutrients such as tannin, alkaloids, saponins, and flavonoids as antioxidants that are responsible for their medicinal use [19, 29]. The plant extracts were able to offer some protection to the liver enzymes related to sub-cellular functions such as plasma membrane and the mitochondrion by reducing the serum activities of ALP, ALT and AST compared to the control. Therefore, the study suggests that both ethanolic bark extracts of *N. latifolia* and *A. cordifolia* may possess relative hepatoprotective activity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Author 1' conceived the study, performed the statistical analysis, interpreted the results and drafted the manuscript. Author 2', Author 3' and Author 5' conduct the study and managed the analyses of the study. Author 4' and Author 6' managed the process of the research. All authors read and approved the final draft of the manuscript.

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