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Research Article

Photochemistry and Toxicity Studies of Aqueous *Nauclea Latifolia* Stem Bark Extract: Effect on Serum Biochemical Markers in Wistar Rats

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Abstract

Bacground: *Nauclea latifolia* is a medicinal plant used in Côte d'Ivoire as a remedy for tooth decay, dental pain and mouth sores. Its consumption can be toxic. *Objective*: The aim of this study was to assess the renal, hepatic and myocardial effects, in order to prevent toxicity. *Materials and methods*: Phytochemistry was carried out by phytochemical screening and thin-layer chromatography. Acute and sub-acute toxicity tests were carried out in accordance with OECD protocols 423 and 407 respectively. Blood samples were taken on days 14 and 28 for analysis of biochemical parameters. Urea was determined using the enzymatic method described by Tietz. Creatinine was determined using the method of Jaffé. Total plasma protein concentration called the "biuret method" developed by Kronh. Alanine aminotransferase activity was determined according to the method recommended by the International Federation of Clinical Chemistry (IFCC). Aspartate aminotransferase (ASAT) activity was determined according to the method of ECCLS. *Results*: Phytochemistry showed the qualitative and expressive presence of polyphenols, flavonoids, tannins, phenolic acids and saponosides. For acute toxicity, somnolence was reported. No mortality or morbidity was observed. The LD50 was greater than 5000 mg/kg. Blood results showed no temporal disturbance in the functioning of biochemical markers of kidney, liver and heart, but some marginal variations were observed. *Conclusion*: The non-toxic effect revealed is proportional to the dose and active substances of the aqueous extract of *Nauclea latifolia* stem bark. This experiment would enable us to formulate improved traditional medicines to help treat oral cavity disorders.

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Keywords

Nauclea Latifolia Stem Bark, Toxicity Studies, Phytochemicals, Biochemical Parameters, Biochemical Markers

1. Introduction

Oral diseases are among the most common non-communicable diseases [20]. These infectious diseases are therefore a major public health concern. Occurring throughout life, they can cause pain and disfigurement, and in some cases prove fatal. Among the most common oral diseases are those of bacterial (dental caries), fungal (candidiasis), viral (herpes) and oral cancer origin [20]. Therapeutically, there are several conventional drugs available. However, some of the conventional drugs on the market and used therapeutically have lost their effectiveness due to resistance phenomena caused by global warming [11]. These problems are compounded by the high cost of conventional medicines and oral care, which is out of reach for low-income and very low-income populations. To achieve this, populations are increasingly turning to medicinal plants for treatment. According to the WHO report [19]. 80% of people in developing countries with low socio-economic status use medicinal plants for their primary care. The use of these medicinal plants in the fight against dental caries, mouth sores and oral cancers is a necessity [8]. However, the stem bark of Nauclea latifolia is one of the medicinal plants used for primary care by people in developing countries. Nauclea latifolia is a member of the Rubiaceae family [3]. It is native to sub-Saharan Africa and possesses active substances for treating numerous pathologies, including oral diseases [5, 8]. The doses used for the various traditional treatments are imprecise and have been the subject of controversy for not being without deleterious effect on the organism [1]. The aim of this study was to evaluate the acute and sub-acute toxic effect of aqueous extracts of Nauclea latifolia stem bark on serum markers of certain vital organs in Wistar rats.

2. Materials and Methods

2.1. Plant Material

The plant material was an obtained plant powder. It consisted of *Nauclea latifolia* stem bark harvested in the Comoé region of Côte d'Ivoire.

2.2. Animal Material

The animal material consisted of non-pregnant Wistar rats (females and males) weighing between 100 and 150g, aged eight to ten weeks.

2.3. Technical Material

This consisted of a sampling sheet, a data collection sheet and laboratory equipment (grinder, mixer, precision balance, stirrer, rotary evaporator, spectrophotometer).

2.4. Plant Identification

Plant identification was carried out at the National Center of Floristics (CNF), using the floras of Aké Assi [2] and Arbonnier [3].

2.5. Preparation of Aqueous Extracts

Preparation consisted in dissolving one hundred grams of dry plant powder in 1000 ml of distilled water, then bringing to the boil over low heat for 20 min. The decoctate was left to cool for 24 hours and filtered once on a sieve (mesh=1mm), then on white cloth, three times on absorbent cotton and once on wartman paper n °1. The filtrate is evaporated in an oven at 60 °C for 48 hours. The dry evaporate, recovered in powder form, constitutes the total aqueous dry extract.

2.6. Characterization of Chemical Compounds

Two methods were used to characterize the chemical compounds in the aqueous extract. The phytochemical screening test, which consists in identifying the chemical compounds in the extract by means of solubility tests, color reactions, precipitation tests and ultraviolet light examinations according to Bidié et al. [7]. The second method is thin-layer chromatography (TLC) to confirm the presence of polyphenols, flavonoids, catechic tannins, phenolic acids and saponosides as the main chemical groups in the aqueous extract of *Nauclea latifolia* stem bark, by separation and identification with spectrophotometric reading.

2.7. Acute and Sub-acute Toxicity Studies

Non-pregnant male and female Wistsar rats, 8 to 10 weeks old, weighing between 100 and 150g were used for the acute and subacute toxicological studies. They were obtained from the pharmacology laboratory of the Faculty of Biology and Pharmaceutical Sciences, Université Félix Houphouët Boigny. Animals were acclimatized to laboratory conditions for 7 days prior to experiments. The rats were maintained at an ambient temperature of 22 to 24 °C, with a 12-h light/dark cycle. During acclimatization, the animals were housed in polycarbonate cages with a standard diet of pellets and tap water.

Food pellets for laboratory animals were purchased from FACI (Cote d'Ivoire).

2.8. Acute Toxicity Study

For acute toxicity, the plant extract was dissolved in distilled water and administered orally to rats (females) of the experimental batch at a single dose of 2000 mg/kg and 5000 mg/kg, respectively at an aqueous solution of 2 ml/kg/rat, while the control batch received only physiological water as vehicle. Rats in the experimental lot were observed relative to the control lot for 24 h, with particular attention paid to the first 4 h and once a day for a period of 14 days. To this end, changes in physical appearance, injuries, pain and signs of disease were monitored once a day during the period [17]. The formula for the volume to be administered by gavage was calculated as follows: V= D.P / C; with V: Volume to be administered by gavage (ml) D: Dose to be administered (mg/Kg body weight); C: Concentration of the extracts stock solution (mg/ml); P: Animal weight (g).

2.9. Subacute Toxicity Study

For subacute toxicity, female and male Wistar rats were treated daily with aqueous extract of *Nauclea latifolia* orally via gavage tube at increasing doses of 50, 100 and 200 mg/kg body weight for 28 days. Animals were anesthetized by inhalation with cooper ether.

2.10. Blood Sampling

Blood was collected from the orbital sulcus on days D0, D14 and D28 [18]. Blood collected in dry Vacutainer tubes was centrifuged at 3500 rpm for 5 min. The sera obtained were used to assess the activity of biochemical serum markers from the heart, including creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and total protein (TP), from the liver, including alanine aminotransferase (ALAT), aspatate aminitransferase (ASAT) and albumin, and from the kidneys, including urea and creatinine.

2.11. Determination

Determination

Urea was determined using the enzymatic method described by Tietz [24] Creatinine was determined using the method of Jaffé [16]. Alanine aminotransferase activity was determined according to the method recommended by the International Federation of Clinical Chemistry (FICC) [6]. Plasma albumin levels were determined by colorimetric assay with bromocresol green using a Kit. LDH is a cytoplasmic enzyme present in all tissues, catalyzing the reversible reduction of pyruvate to lactate according to the Henry reaction [9]. The rate of decrease in NADH concentration, directly proportional to LDH activity in the sample, was measured at 340 nm. This is a quantitative assay of total plasma protein concentration called the "biuret method" developed by Kronh [15]. CPK is a dimeric enzyme composed of two subunits: the M subunit and the B subunit. These subunits are combined to form 3 distinct isoenzymes: CPK-BB, CPK-MB and CPK-MM. The modified CPK-NAC reagent contains a polyclonal antibody which inhibited all CPK-MM activity and half of CPK-MB activity. Only the activity of the uninhibited B subunit, representing half of CPK-MB activity, was measured. This method reveals that CPK-BB activity in plasma is negligible. LDH is a cytoplasmic enzyme present in all tissues and catalyzes the reversible reduction of pyruvate to lactate according to the Henry reaction [9]. The rate of decrease in NADH concentration, directly proportional to LDH activity in the sample, was measured at 340 nm. This is a quantitative assay of plasma total protein concentration called the "biuret method" developed by Kronh [15]. Alanine aminotransferase activity was determined according to the method recommended by the International Federation of Clinical Chemistry (IFCC) [6]. Aspartate aminotransferase (ASAT) activity was determined according to the method of ECCLS [10] and the recommendations of the International Federation of Clinical Chemistry (FICC). Plasma albumin levels were determined by colorimetric assay with bromo-cresol green using a Kit.

2.12. Statistical Analysis

Statistical Analysis

Statistical analysis of significance tests was performed for comparisons of 2 means by the T test or Mann-Whiney/Wilcoxon, and for comparisons of more than 2 means by the Anova or Kruskal-Wallis test.

3. Results

The animals were treated with aqueous extract of *Nauclea latifolia* stem bark at different doses of 50, 100, and 200 mg/kg body weight. The results showed dose-response effects that did not produce any disturbance over time in the functioning of serum biochemical markers of the kidneys, liver, and heart of Wistar rats, but some marginal variations were observed, as shown in the tables below.

The three tables show myocardial values in red, hepatic values in yellow and nephrotic values in green. Table 1 shows the mean values of all biochemical parameters measured in treated and untreated rats. Table 2 shows the mean values of biochemical parameters in rats as function of treatment dose. Table 3 shows the mean values as function treatment duration.

Table 1. *Average values of biochemical parameters in treated and untreated rats.*

Parameters	Mean ± SD	Median	Minimum	Maximum	P test
CPK					
NT	2942,91 ± 1364,9	2630,5	1638	5989	0,00
TT	957,85 ± 397,3	855	533	2155	(S)
LDH					
NT	4973 ± 7891,9	2846	1825	2999	0,10
TT	2477,05 ± 504,7	2461,5	1610	3564	(NS)
Protein					
NT	76 ± 11,64	71,50	61	107	0,001
TT	64,05 ± 7, 61	63,50	47	81	(S)
TGO					
NT	327,58 ± 88,20	315	233	514	0,08
TT	255,35 ± 120,49	228	96	719	(NS)
TGP					
NT	88,16 ± 30,17	79,50	55	142	0,00074
TT	132,0 ± 32,92	125,50	81	221	(S)
Albumin					
NT	42,91 ± 10,60	44	25	60	0,06
TT	49,10 ± 6,47	49	30	57	(NS)
UREA					
NT	0,37 ± 0,069	0,37	0,28	0,47	0,27
TT	0,40 ± 0,080	0,43	0,23	0,50	(NS)
CREAT					
NT	4,33 ± 0,8876	4,00	3,00	6,00	0,74
TT	4,25 ± 0,5501	4,00	3,00	6,00	(NS)

SD: Standard Deviation
NT: Not Treated
TT: Treated
P significant: Mann-Whitney test
P not significant: T Test

Table 2. *Biochemical parameter values according to treatment dose.*

Parameters		Mean ± sd	Median	Minimum	Maximum	P test
CPK	0	2942,9 ± 1364,9	2630	1638	5989	0,0001 (S)
	50	886 ± 339,32	790,50	533	1470	
	100	809,57 ± 162,97	842,0	569	1066	
	200	1167,71 ± 543,27	1136	596	2155	
LDH	0	4973 ± 7891,1	2846	1825	2999	0,34 (NS)

Parameters		Mean ± sd	Median	Minimum	Maximum	P test
	50	2426 ± 469,05	2493	1742	2975	
	100	2376,14 ± 431,3	2403	1610	2865	
	200	2621,71 ± 631,2	2675	1956	3564	
	0	76 ± 11,64	71,50	61	107	
Protein	50	65,33 ± 6,43	66	55	73	0,013 (S)
	100	65,57 ± 7,61	65	57	81	
	200	61,42 ± 8,88	61	47	71	
	0	327,58 ± 88,20	315	233	514	
TGO	50	226,66 ± 37,72	222	190	296	0,0011 (S)
	100	229,14 ± 36,27	223	190	300	
	200	306,14 ± 197,08	264	96	719	
	0	88,16 ± 30,17	79,5	55	142	
TGP	50	139,16 ± 24,02	136	114	172	0,009 (S)
	100	129,14 ± 28,42	123	97	172	
	200	128,71 ± 45,51	124	81	221	
	0	42,91 ± 10,60	44	25	60	
Albumin	50	50,50 ± 4,84	48,50	45	57	0,3191 (NS)
	100	47,0 ± 9,59	50,00	30	57	
	200	50 ± 3,65	49	46	55	
	0	0,37 ± 0,069	0,37	0,28	0,47	
UREA	50	0,36 ± 0,09	0,34	0,26	0,50	0,31 (NS)
	100	0,41 ± 0,08	0,44	0,23	0,50	
	200	0,42 ± 0,05	0,44	0,33	0,49	
	0	4,3 ± 0,88	4,0	3,0	6,0	
CREAT	50	4,1 ± 0,40	4,0	4,0	5,0	0,46 (NS)
	100	4 ± 0,57	4,0	3,0	5,0	
	200	4,5 ± 0,53	5,0,	4,0	5,0	

SD: Standard Deviation
P significant: Kruskal-Wallis H test
P not significant: T Anova
The different administrated doses:
0: No administrated dose
50: dose of 50 mg/kg
100: dose of 100 mg/kg
200: dose of 200 mg/kg

Table 3. *Biochemical parameter values according to the duration of treatment.*

Parameters	Mean ± sd	Median	Minimum	Maximum	P test
CPK					

Parameters	Mean ± sd	Median	Minimum	Maximum	P test
0	2942,9 ±1364,9	2630	1638	5989	
14	847,08 ±441,24	699	533	2155	0,000 (S)
28	1124 ±264,41	1101	842	1470	
LDH					
0	4973 ± 7891,9	2846	1825	2999	
14	2392 ± 570,50	2354	1610	3564	0,17 (NS)
28	2604 ± 386,27	2744	1950	2975	
Protein					
0	76 ± 11,64	71,5	61	107	
14	67 ± 753	66,5	55	81	0,001 (S)
28	59,62 ± 5,57	61,5	47	64	
TGO					
0	327,58 ±88,20	315	233	514	
14	293,16 ±138,37	242	222	719	0,007 (S)
28	198,62 ± 56,50	197	96	306	
TGP					
0	88,16 ± 30,17	79,50	55	142	
14	124,91 ± 19,58	125,50	94	162	0,008 (S)
28	142,62 ± 46,05	137,0	81	221	
Albumin					
0	42,91 ± 10,60	44	25	60	
14	48,66 ± 7,01	49	30	56	0,14 (NS)
28	49,75 ± 5,99	49	39	57	
UREA					
0	0,37 ± 0,069	0,37	0,28	0,47	
14	0,37 0,094	0,40	0,23	0,50	0,17 (NS)
28	0,43 0,033	0,44	0,39	0,43	
CREAT					
0	4,3 ± 0,88	4	3	6	
14	4,25 ± 0,62	4	3	5	0,94 (NS)
28	4,25 ± 4,46	4	3	5	

SD: Standard Deviation
P significant: Kruskal-Wallis H test
P not significant: T Anova
0: No treatment
14: Treatment at day 14
28: Treatment at day 28

4. Discussion

Myocardial assessment

The results of myocardial biomarker measurements show a non-significant difference ($p > 0.05$) in the mean serum levels of total protein, creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) in rats from the experimental group treated with different doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg compared to those of rats in the control group, followed by no change in LDH on day 28 (Tables 1-3).

LDH and CPK are enzymes used as sensitive markers to assess the degree of myocardial necrosis. CPK is an enzyme found mainly in muscles, which is involved in energy storage through a mechanism called creatine phosphorylation. LDH, on the other hand, is an enzyme found mainly in plasma. LDH is an indicator of a disturbance in the cell's energy balance, leading to the accumulation of this enzyme followed by cell death [12]. The results of the myocardial biomarker assay show a non-significantly different ($p > 0.05$) decrease in the mean values of serum total protein, creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) in rats from the experimental group treated with different doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg compared to those in the control group, followed by no change in LDH on day 28. Ultimately, the aqueous extract of *Nauclea* stem bark has cardioprotective properties.

Liver function test

The results show that the aqueous extract of *Nauclea latifolia* stem bark significantly reduced serum aspartate aminotransferase (AST or TGO) levels, but a significant increase in alanine aminotransferase (ALAT or TGP) and albumin levels was detected in the rats in the experimental group compared to the control group (Tables 1-3).

The results of this study show that the aqueous extract of *Nauclea latifolia* stem bark significantly reduced serum aspartate aminotransferase (AST or TGO) levels. This result differs from those of some author's [4], who reported an increase in AST. Furthermore, our results corroborate those of Arise et al [4], who showed an increase in ALAT. The authors concluded that *Nauclea latifolia* extract does not negatively interfere with amino acid metabolism in the liver, but can cause destruction of liver tissue in a time- and concentration-dependent manner when higher doses (200, 400, 600, and 800 mg/kg) are used [24]. This could explain the increase in serum ALAT and, contrary to the decrease in these parameters, corresponds to the results of the study by Kouadio et al [14]. However, Kouadio et al used doses (1.8, 18, and 180 mg/kg body weight) that were partially similar to those used in the present study. Analysis of the results of the present study shows that administration of the aqueous extract of *Nauclea latifolia* stem at different doses (50, 100, and 200) for 28 days does not cause significant damage to liver tissue based on ASAT and ALAT serum levels.

Albumin is a protein found in abundance in the blood and produced by the liver. Albumin in plasma acts as an antioxidant, which is why its increase detected in this study would contribute to reducing the proliferation of cancer cells. This result confirms that the aqueous extract of *Nauclea latifolia* stem, which has antioxidant activity, would be able to further activate albumin in its antioxidant role.

Nephrotic Balance

The results presented in the graphs below (Tables 1-3) show that the aqueous extract administered to rats in the experimental group at increasing doses significantly altered serum urea activity, followed by stabilization of creatinine activity compared to that of rats in the control group.

Most of the time, these urea and creatinine compounds are filtered by the kidneys with little or no tubular reabsorption [13]. Any phenomenon or substance capable of altering these various renal functions inevitably leads to changes in the plasma concentrations of these metabolites [21]. Our results are similar to those of Kouadio et al [14], who showed that high doses of plant extract (18 and 180 mg/kg) for 28 days may be indicative of impaired renal evocative and excretory functions [22, 23] caused by N. latifolia extract. Unlike previous analyses, in the present study, the extract did not cause any changes in creatinine function in the control group rats compared to those in the control group rats.

5. Conclusion

The phytochemistry of the aqueous extract of *Nauclea Latifolia* showed the qualitative and expressive presence of polyphenols, flavonoids, tannins, phenolic acids, and saponosides. For acute toxicity, drowsiness was reported. No mortality or morbidity was observed. The LD50 was greater than 5000 mg/kg. Blood test results showed no disruption in the functioning of biochemical markers of the kidneys, liver, and heart over time, but some marginal variations were observed. The non-toxic effect revealed is proportional to the dose and active substances in the aqueous extract of *Nauclea latifolia* stem bark. This experiment could lead to the formulation of improved traditional medicines to help treat oral cavity conditions.

Abbreviations

OECD	Organisation for Economic Co-operation and Development
IFCC	International Federation of Clinical Chemistry
ASAT or TGO	Aspartate Aminotransferase
ECCLS	European Council for Clinical and Laboratory Standardization
WHO	World Health Organisation
CNF	National Center of Floristics
LD50	Lethal Dose 50
TLC	Thin-layer Chromatography
CPK	Creatine Phosphokinase

LDH	Lactate Dehydrogenase
TP	Total Protein
ALAT or TGP	Alanine Aminotransferase

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Conflicts of Interest

The authors declare no conflicts of interest.

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