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Acute and Subacute Toxicity of *Lannea acida* A. Rich Hydroethanolic Trunk Bark Extract on *Wistar* rats and *NMRI* Mice

Abdelaziz Koussoube*[1], Filkpièrè Léonard DA[1,2], Basile Tindano[1], Tééganimbé Jean Luc Kabore[1], Elisabeth Ouedraogo[1] and Balé Bayala[1]

[1]Department of Animal Biology and Physiology, Animal Physiology Laboratory, University Joseph KI-ZERBO, Ouagadougou, Burkina Faso.
[2]Faculty of Science and Technology, Life and Earth Sciences Laboratory, University Norbert Zongo, Koudougou, Burkina Faso.
*Corresponding Author Email: abdelaziz_koussoube@ujkz.bf

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Lannea acida **is a medicinal plant used in treatments for diabetes and infertility. This study's purpose was to evaluate the safety of** ***Lannea acida*** **trunk bark. Phytochemical screening was performed on the hydroethanolic extract of** ***Lannea acida*** **trunk bark. Acute innocuity was assessed in female** ***NMRI*** **mice with a limited dose of 2000 mg/kg bw (body weight). Subacute safety was investigated too in female** ***Wistar*** **rats throughout 28 days by daily administration. Four groups of six rats were formed and received distilled water and the extract at 40, 100, and 200 mg/kg bw respectively. Two other groups served as satellites and received water and a higher dose. Phytochemical screening revealed the presence of tannins, polyphenols, coumarins, alkaloids, triterpenes, and steroids. The quantitative assay revealed polyphenols, flavonoids, and tannins levels 649.77±0.36 mg EAG/g, 7.46±0.13 mg EQ/g, 79.71 ± 2.78 mg EAT/g, respectively. The extract administered as a single dose did not cause any mortality. The LD50 is greater than 2000 mg/kg. At repeated doses for 28 days, the extract did not show any significant (p>0.05) variation in animal weight. No dose of extract caused a significant (p>0.05) variation in relative organ weights. Blood counts showed no significant (p>0.05) variation. The following biochemical parameters did not vary significantly (p>0.05): Urea, creatinine, total bilirubin and direct bilirubin, triglycerides, total cholesterol, AST, and ALT. The liver functions of the rats were not affected. This study shows that the hydroethanolic extract of** ***Lannea acida*** **trunk bark is not toxic for the single and medium term.**

Keywords: Acute Toxicity; Hydroethanolic Extract; *Lannea Acida*, LD50; Subacute Toxicity.

For thousands of years, African societies have traditionally used medicinal plants to alleviate disease. Recent years have seen the emergence of non-communicable diseases including diabetes, which is becoming increasingly prevalent worldwide. [1] In 2016, WHO (World Health Organization) estimates that 41 million people worldwide die from chronic diseases. Deaths caused by diabetes were estimated at 1.6 million people. The mortality rate was higher in low-and middle-income countries between 2000 and 2016. [2] As with other non-communicable diseases, diabetes remains a public health concern in terms of complications. Medicinal plants remain an alternative form of healthcare due to their availability, efficacy, and low cost.

Ethnobotanical studies have shown that certain plants can help combat diabetes and its effects on fertility, which is one of the complications of diabetes. One of these plants is *Lannea acida.* [3,4] The genus *Lannea* belongs to the *Anacardiaceae* family and contains 36 species. Its range extends from tropical and southern Africa, the southern Arabian Peninsula, and the Indian subcontinent to southern China and Indochina. [5] Ethnobotanical surveys have shown that the species *Lannea acida* is used empirically to treat diabetes [3,6] and infertility. [4] This species relieves digestive disorders such as diarrhea [7]. It treats oral and dental infections. [8] Previous pharmacological studies have highlighted certain biological properties of *Lannea acida* trunk bark. These include uterotonic [9], antimicrobial and antioxidant, [10–12] anti-inflammatory and analgesic [13], and anti-osteoporotic effects. [14] *Lannea acida* improves the emissive phase of ejaculation. [15] A study on fertility and testosterone levels in male rats showed that the methanolic extract of the stem bark of *Lannea acida* improves sperm count, morphology, motility, and serum testosterone levels. [16] Few data are available on the anti-diabetic activity of *Lannea acida*. Therefore, the purpose of the present study was to contribute to the evaluation of the safety of *Lannea acida* trunk bark, a plant used traditionally to treat diabetes and infertility.

MATERIALS AND METHODS

Plant materials

Plant material consisted of bark from the trunk of *Lannea acida* A. Rich. collected between 6 and 10 am. in the province of Sanmatenga, commune of Mané located at 90 Km from Ouagadougou. The barks were washed and dried in the laboratory under ventilation without sunlight and dishes. They were ground into a very fine powder and stored in plastic in a dry place.

Animal materials

The tests were carried out on *NMRI* (Naval Medical Research Institute) mice and *Wistar* rats, all females. They weighed an average of 32 g and 122 g respectively. The mice were 9 weeks old and the rats were 10 weeks old. The animals were provided by the University Joseph KI-ZERBO animal house. They were reared at a temperature of 22 ±3ºC, a relative humidity of 50 ±10%, and a lighting duration of 12h per day. The animals had access to feed containing 29% protein. All the experiments were achieved according to the animal ethics committee of UJKZ (University Joseph KI-ZERBO) under the number CE-UJKZ/2023-14.

Extraction

The RMC (residual moisture content) of the plant material was determined by the drying. [17] It was 6.28% ± 0.05.

One hundred grams (100 g) of plant powder was added to 1000 ml of an 80% (v/v) hydroethanolic solution. The whole has been homogenized for 72 hours in the laboratory at ambient temperature. The macerate was then filtered through No. 5 of the Wahatman paper. The filtrate obtained was evaporated in a rotary evaporator at 50°C under reduced pressure. The concentrated filtrate was frozen and then lyophilized to obtain the HEELA (hydroethanolic extract of *Lannea acida*). The extraction yield was 19.27% ±0.41.

Phytochemical screening

Phytochemical characterization aimed to identify the mayor chemical compounds of HEELA using a previous method described. [18] For this purpose, 100 mg of the extract was diluted in 10 ml of diluent (ethanol or methanol) to serve as a stock solution for phytochemical screening. Several tests were carried out to detect the presence or absence of certain constituents considered to be bioactive in the hydroethanolic extract of *Lannea acida* trunk bark. The ferric chloride test was used to characterize tannins and polyphenols. The Shibata test was used to characterize flavonoids. The Libermann-Bürchard test was used for sterols and triterpenes. The foam index was used to characterize saponosides. The Dragendorff reagent (potassium tetra iodo bismutate) was used to characterize alkaloids. Coumarins were characterized by the ammonium hydroxide test based on the appearance of green or blue fluorescence under a 365 nm UV (ultraviolet) lamp.

Quantitative phytochemical assays

These consisted of assays of polyphenols, flavonoids and tannins contents. The Folin-Ciocalteu colorimetric method was used to assess total polyphenolics. [19] She evaluated all the phenolic compounds which are reducers of the phospho-molybdotungstic reagent (Folin-Ciocalteu

reagent). The levels of total phenolics content were obtained by standard curve extrapolation obtained through a series of dilutions with distilled water and gallic acid (0-200 mg/L). Absorbance measured at 760 nm. Total polyphenol content was reported as mg EGA (milligram Equivalent Gallic Acid) per dry extract gram.

The Flavonoid content of the HEELA was quantified using the method of aluminum trichloride with a few adjustments. [20] A quercetin reference curve standard has been established. After 15 min, the absorbance was read at 415 nm. Total flavonoid was reported as mgEQ/g (milligram Equivalent Quercetin per dry extract gram).

The tannin content was quantified according to a previous method with a few adjustments. [21] The quantitative value has been reported as mg ETA/g (milligram Equivalent Tannic Acid per dry extract gram) using the calibration curve for tannic acid.

Acute innocuity

The acute innocuity study was carried out according to OECD [22] (Organization for Economic Cooperation and Development) directive 423. Two groups of three mice were established, with an average weight of 32 g. The mice were fasted for 4 hours. The group 1 and 2 have a unique dose by gavage of water and the HEELA at a dose of 2000 mg/kg bw (milligram per kilogram per body weight), respectively. Observations were made during the 4 first hours after gavage and then continuous for 14 days. Observations were made on mobility, hair appearance, eye color, mortality, and somnolence to identify where possible toxicity effects.

Subacute innocuity

The subacute toxicity study was conducted by OECD [23] (Organization for Economic Cooperation and Development) directive 407. In this study, six groups of six female *Wistar* rats were formed, so a total of 36 rats. The control group has taken delivery of the distilled water 10ml/kg bw. The three test groups each have taken delivery of the HEELA at doses of 40, 100, and 200 mg/kg bw. The satellite group received distilled water and the HEELA at 200 mg/kg bw. These groups were observed remained under observation for 14 days after treatment to observe the reversibility of toxicity signs. Test doses were administered daily by gavage as a single dose for 28 consecutive days. Rats were fed and watered ad libitum and weighed every 7 days to determine dose-related body-weight gain. The animals were carefully observed at the same time for any signs of toxicity. After the last day of treatment, rats were deprived of food overnight, anesthetized with lidocaine 20% and ketamine 50 mg/kg, and then sacrificed. [24] Blood was collected by cardiac aspiration, in EDTA (Ethylene Diamine Tetraacetic Acid) and dry tubes for the determination of hematological and biochemical parameters respectively. The animals were autopsied and certain organs like the spleen, the heart, the kidneys, the liver, the lungs, and the pancreas were removed and weighed. Their relative weights were calculated. Biochemical parameters were determined using ATLAS MEDICAL assay kits. Urea was determined by the calorimetric-enzymatic test, creatinine by the Jaffé kinetic calorimetric method, total bilirubin and direct bilirubin by the calorimetric method, triglycerides GPO-POD (glycerol-3-phosphate-peroxidase) Liquid, cholesterol (CHOD/POD (Cholesterol oxidase/ Peroxidase) method), ALAT (alanine aminotransferase), ASAT (aspartate aminotransferase).

Histopathological examination of the liver and kidneys

Liver and kidney samples from the control lot and the lot treated with the highest dose of the extract (200mg/kg) were preserved in 10% formalin. The samples underwent paraffin embedding after a series of dehydration in alcohol baths. Sections were cut using a microtome, stained with hematein and eosin, and mounted on slides covered with EUKIT glue. The slides were observed using a Motic BA310E LED light microscope.

Statistical analysis

The data were collected by Excel software version. 2016. Graph Pad version 8.4.3 was used to perform the statistical analyses of the data. The data were shown as Mean ± Standard Deviation in tables and graphs. ANOVA (One-way analysis of variance) was applied to compare means between groups. The Tukey Test was used to define the difference between two values. The degree of significance was considered to be P-value <0.05.

RESULTS

Phytochemical screening

Characterisation tests on the hydroethanolic extract of *Lannea acida* showed the existence of compound such as tannins and phenols, coumarins, alkaloids, triterpenes and steroids (Table I).

Phytochemical content of the extract

The table II shows the contents of the polyphenols, flavonoids and tannins. The content was estimated to be 649.77±0.36 mg EGA/g for total polyphenols. Moreover, the total flavonoids was estimated to 7.46±0.13 mg EQ/g. And the total tannins was estimated 79.71 ± 2.78 mg ETA /g.

Acute innocuity

No signs of toxicity (normal coat, no trembling, no aggressiveness) were observed 4 h after *Lannea acida* trunk bark hydroethanolic extract administration at 2000 mg/kg bw. Also, no death was noted after 14 days after treatment.

Subacute innocuity

Effects of hydroethanolic extract of *Lannea acida* trunk bark on weight evolution in rats

During the four weeks of observation, no significant ($p>0.05$) change in body weight was noted between the different test groups of animals. Similarly no significant ($p>0.05$) change was noted between the satellite groups (Figure 1).

Effect of the extract on relative organ weights

No dose of HEELA produced a significant ($p>0.05$) change in relative organ weights in comparison with the blank control group. A non-significant ($p>0.05$) augmentation in relative liver weight was noted at all doses compared with the blank control. Moreover, at 40 and 100 mg/kg bw, relative lung and spleen weights showed a non-significant ($p>0.05$) diminution in comparison with the blank control. A diminution of no significance ($p>0.05$) was observed in relative kidney weight at 40 mg/kg bw. On the other hand, at 40 mg/kg bw there was a non-significant ($p>0.05$) diminution in the relative weights of the pancreas and heart in comparison with the blank control. At 200 mg/kg bw, the relative weights of the lung and heart showed non-significant augmentation ($p>0.05$) in comparison with the blank control. In satellite groups, the relative weights of the liver, pancreas, and lungs showed non-significant ($p>0.05$) diminution in comparison with the control. In addition, a non-significant ($p>0.05$) augmentation was noted in the relative weights of the heart and spleen in comparison with the control. (Table III).

Table 1. Chemical compounds identified in the hydroethanolic extract of *Lannea acida* trunk bark

Compounds	Tanninsand phenols	Flavonoids	Coumarins	Alkaloids	Tri-terpenes and steroids	Saponosides
Hydro-Ethanolic Extract	+	-	+	+	+	-

Legend : (+) : Detected compound, (-) : No detected compound

Table 2. Quantitative determination of polyphenols, flavonoids and tannins

Phytochemical classes	*Lannea acida* contents
Polyphenols	649.77±0.36 mg EGA/g
Flavonoids	7.46±0.13 mg EQ/g
Tannins	79.71 ± 2.78 mg ETA/g

Legend :
mg EGA: milligram Equivalent Gallic Acid
mg EQ : milligram Equivalent Quercetin
E TA: milligram Equivalent Tannic Acid
g: gram
mg : milligram

Effects of *Lannea acida* hydroethanolic extract on hematological parameters

Hematological analyses showed no significant variation in the levels of red blood cells, hemoglobin, hematocrit, leukocytes, neutrophils, lymphocytes, and platelets (Table IV). The red blood cells, hemoglobin, hematocrit, and platelets count showed a non-significant ($p>0.05$) increase at all doses of HEELA in comparison with the blank control. Leucocyte count showed a non-significant ($p>0.05$) increase at 40 and 200 mg/kg bw in comparison with blank control. In addition, neutrophil percentage was increased non-

significantly (p>0.05) at 100 and 200 mg/kg bw in comparison to blank control. A non-significant (p>0.05) decrease was noted at 40 mg/kg bw HEELA compared to blank control. The percentage of lymphocytes showed a non-significant (p>0.05) decrease at the different doses of HEELA compared to the blank control. Furthermore, platelets were increased non-significantly (p>0.05) at all HEELA doses. For the satellite groups, red blood cells, hemoglobin, hematocrit, and leucocytes showed a non-significant (p>0.05) decrease compared with the control. A non-significantly increase (p > 0.05) was noted in platelet and lymphocyte levels.

Effects of *Lannea acida* hydroethanolic extract on Biochemical Parameters

No significant variation was observed in the biochemical parameters of ALAT, ASAT, direct and total bilirubin, total cholesterol, triglycerides, urea, and creatinine when comparing the different doses of HEELA 200 mg, 100 mg, 40 mg/kg bw to the blank control and the satellite batches to each other (Table V). The extract at all doses showed a non-significantly decrease (p>0.05) in ASAT, ALAT, and creatinine levels in comparison with the blank control. However, a non-significantly increase (p>0.05) was observed in total cholesterol levels at all doses in comparison with the control. Furthermore, total bilirubin levels decreased non-significantly (p>0.05) of the HEELA at 40 and 100 mg/kg bw, and direct bilirubin levels at 40 and 200 mg/kg bw in comparison to blank control. The HEELA induced a non-significantly increase (p>0.05) in total bilirubin levels at the dose of 200 mg/kg bw. Similarly, it showed a non-significantly increase (p>0.05) in the level of direct bilirubin at the dose of 100 mg/kg bw in comparison with the blank control. At the different doses of the HEELA, triglyceride, and urea levels showed no significant variation (p>0.05) in comparison with the blank control. For satellites, the HEELA induced a non-significant increase (p>0.05) in ALAT, total bilirubin, and triglyceride levels compared with the blank control. For satellite groups, a non-significant decrease (p>0.05) was noted at 200 mg/kg bw in the AST, direct bilirubin, total cholesterol, and creatinine levels in comparison with the control.

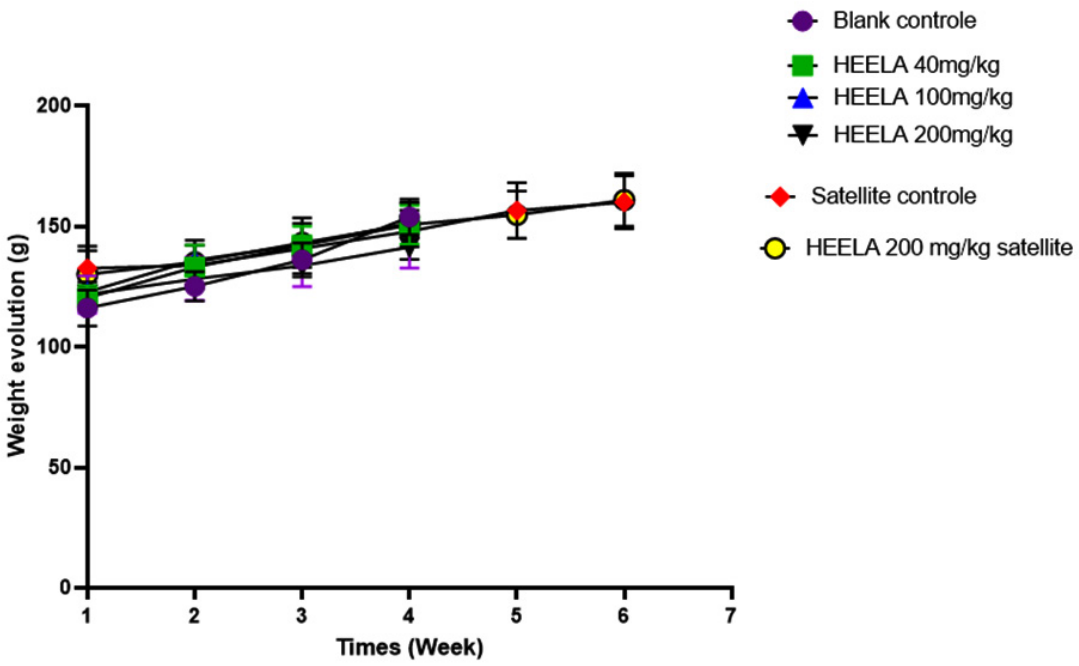

Fig. 1. Effect of hydroethanolic extract of *Lannea acida* trunk bark on body weight evolution in rats
Values are reported as mean ± standard error; n = 6.
Control: distilled water
Extract: hydroethanolic extract of *Lannea acida* trunk bark

Table 3. Effects of *Lannea acida* hydroethanolic extract on relative organ weights

Relative weights	Treatment (mg/kg)					
	Blank control	HEELA 40	HEELA 100	HEELA 200	Control Satellite	Satellite positive
Liver	3.50 ± 0.10	3.68 ± 0.10	3.54 ± 0.16	3.67 ± 0.09	3.71 ± 0.10	3.70 ± 0.08
Kidney	0.59 ± 0.02	0.58 ± 0.04	0.59 ± 0.04	0.59 ± 0.04	0.60 ± 0.02	0.60 ± 0.02
Spleen	0.28 ± 0.02	0.27 ± 0.03	0.27 ± 0.03	0.28 ± 0.02	0.27 ± 0.02	0.28 ± 0.03
Lungs	0.82 ± 0.04	0.80 ± 0.04	0.80 ± 0.03	0.84 ± 0.02	1.11 ± 0.17	0.993 ± 0.11
Heart	0.40 ± 0.01	0.41 ± 0.02	0.40 ± 0.02	0.43 ± 0.02	0.43 ± 0.02	0.44 ± 0.02
Pancreas	0.46 ± 0.03	0.47 ± 0.01	0.46 ± 0.03	0.46 ± 0.03	0.53 ± 0.04	0.51 ± 0.02

Values were reported as mean ± standard error; n = 6.
Legend :
HEELA 40: Hydroethanolic extract of *Lannea acida* 40mg/kg body weight
HEELA 100: Hydroethanolic extract of *Lannea acida* 100mg/kg body weight
HEELA 200: Hydroethanolic extract of *Lannea acida* 200mg/kg body weight

Table 4. Effects of *Lannea acida* hydroethanolic extract on hematological parameters

Hematological parameters	Treatments (mg/kg)					
	Blank control	HEELA 40	HEELA 100	HEELA 200	Control satellite	Positive satellite
Red blood cells ($x10^6$ /µL)	6.34±0.39	6.36±0.17	6.38± 0.24	6.37± 0.16	8.02±0.15	7.52±0.39
Haemoglobin (g/dL)	13.15±0.38	13.57±0.55	13.4 ± 0.40	13.55±0.51	14.05±0.05	14±0.21
Haematocrit (%)	34.62±0.43	34.75±0.31	34.8 ± 0.60	34.8 ± 0.43	45.86±0.62	44.85±0.70
Leukocytes ($x10^3$ / µL)	4.17±0.11	4.18 ± 0.10	4.17 ± 0.16	4.41 ± 0.17	8.28 ± 0.58	8.01±0.68
Neutrophils (%)	29.12±0.58	29.08±0.52	28.92±0.50	29.17±0.78	29.76±1.03	30.38±1.01
Lymphocytes (%)	46.74±0.18	46.51±0.10	46.54±0.08	46.54±0.13	71.54±0.93	71.68±0.17
Platelets ($x10^3$ /µL)	1034±18.16	1049±14.21	1040±13.03	1036.5±24.79	1248±10.25	1259 ± 17.83

Values were expressed as averages ± standard error; n = 6.
Legend :
HEELA 40: Hydroethanolic extract of *Lannea acida* 40mg/kg body weight
HEELA 100: Hydroethanolic extract of *Lannea acida* 100mg/kg body weight
HEELA 200: Hydroethanolic extract of *Lannea acida* 200mg/kg body weight
10^6: 1000000
10^3: 1000
% : Percent
dL: Deciliter
µL: Microliter

A non-significantly increase ($p>0.05$) was noted at 200 mg/kg bw in ALT, total bilirubin, and triglyceride levels compared to the control. The urea level did not vary significantly ($p>0.05$) at 200 mg/kg bw of the extract in comparison with the control.

Histopathological examination of the liver and kidneys

Figure 2 shows histological sections of the liver and kidneys of control rats and rats treated with the highest dose (200mg/kg bw) of HEELA. Examination of the sections showed normal organ

Table 5. Effects of *Lannea acida* hydroethanolic extract on biochemical parameters

Biochemical parameters	Treatments (mg/kg)					
	Blank control	HEELA 40	HEELA 100	HEELA 200	Control satellite	Positive satellite
ALT (UI/L)	73,81±2,4	73,38±2,67	72,03±2,68	72,75±3,16	75,64±1,01	76,49±1,01
AST (UI/L)	226,3±5,78	223,38±3,98	224,89±4,06	225,6±4,04	225,3±4,96	224,4±5,93
Direct bilirubin (µmol/L)	1,74±0,06	1,72±0,04	1,76±0,04	1,72±0,06	1,68±0,05	1,66±0,07
Total bilirunin (µmol/L)	6,45±0,12	6,31±0,26	6,33±0,12	6,50±0,10	6,31±0,25	6,43±0,25
Total cholesterol (mmol/L)	7,49±0,31	7,56±0,22	7,52±0,20	7,65±0,09	8,35±0,27	8,16±0,33
Triglycerides (mmol/L)	1,13±0,01	1,13±0,01	1,13±0,01	1,13±0,01	1,09±0,046	1,13±0,03
Urea (mmol/L)	3,85±0,004	3,85±0,0002	3,85±0,0009	3,85±0,0057	3,88±0,0002	3,88±0,0001
Creatinine (µmol/L)	180,19±0,24	179,84±0,23	179,85±0,18	179,78±0,35	180,5±2,30	179,1±2,28

Values were expressed as mean ± standard error; n = 6.
Legend :
HEELA 40: Hydroethanolic extract of *Lannea acida* 40mg/kg body weight
HEELA 100: Hydroethanolic extract of *Lannea acida* 100mg/kg body weight
HEELA 200: Hydroethanolic extract of *Lannea acida* 200mg/kg body weight
ALT : Alanine aminotransférase
ASAT : Aspartate aminotransférase
UI: International unit
L: Liter
mmol: Millimole
µmol: Micromole

structure in the control lot and the lot treated with the highest dose of the extract.

DISCUSSION

The scope of the study was to contribute to the safety assessment of the safety of *Lannea acida* trunk bark, a plant used traditionally to treat diabetes and infertility. No effects of acute toxicity were detected after the HEELA was administered at a dose of 2000 mg/kg bw for 14 days post-dosing. Also, no death was detected during a period of exposure of 14 days. Our results are in agreement with those [7] after administration of 3000 mg/kg bw of *Lannea acida* trunk bark aqueous extract. According to Olusola et al [25] methanolic extract of *Lannea acida* trunk bark is non-toxic at 5000 mg/kg bw. This was after single-dose treatment with 5000 mg/kg bw and observation for 7 consecutive days. These findings show that the HEELA would be non-toxic, hence its use in traditional medicine against diabetes and infertility. *Lannea acida* trunk bark extract could therefore be classified as category 5 according to the OECD [22] Globally Harmonised Classification System. The LD 50 50 (lethal dose) of the extract would be higher than 2000 mg/kg bw. Further investigations are required to determine the precise lethal dose. After 28 days of treatment, the HEELA produced no effect on body weight in rats at the various doses. This could be explained by a physiological adaptation of the animals. [26] The relative weights of the organs including liver, heart, lungs, pancreas, spleen, and kidneys weren't significantly different between the control and test groups. Analysis of ALAT and ASAT transaminases showed no significant

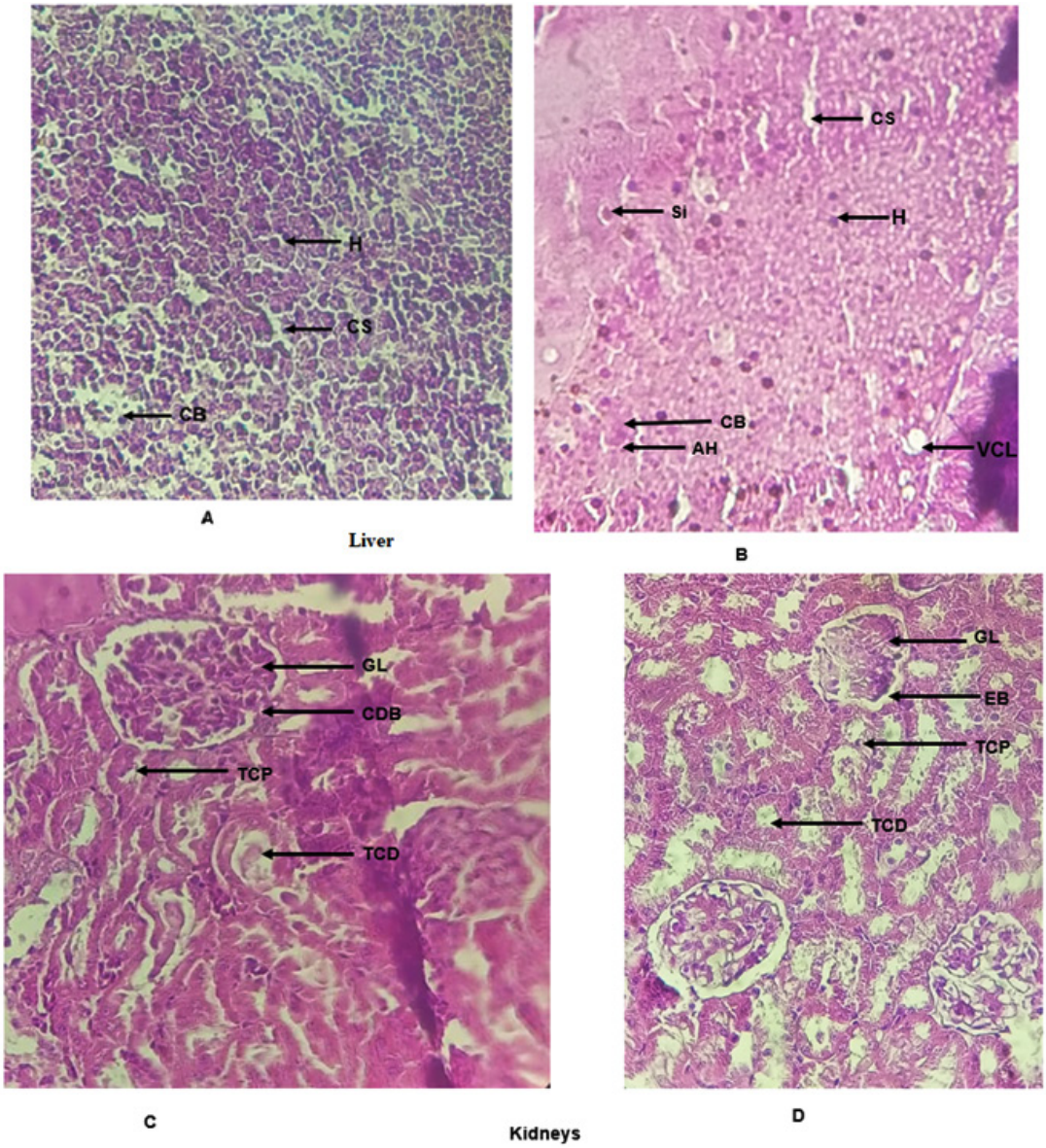

Fig. 2. Sections of liver and kidney from the control groups and the 200mg/kg extract (H & E, G= 400)
Legend:
H & E: Hematoxylin and eosin
G: Magnification
A: Liver of the control batch; B: Liver of the batch treated with 200mg/kg bw; C: Kidney of the control batch and D: Kidney of the batch treated with 200mg/kg bw.
CB: biliary canaliculus, CS: canaliculus, H: hepatocytes, VCL: centrolobular vein, Si: sinusoid, AH: hepatic artery, GL: glomerulus, CDB: Bowman's capsule, ES: Bowman's espace, TCD: distal contoured tubule, TCP: proximal contoured tubule.

change. ALAT is found in hepatocytes, in the cytoplasm, but also muscle and the kidney. ASAT, on the other hand, is more widely distributed in the liver, as well as in the heart, skeletal muscle, kidneys, and brain. [27] No changes were observed in total or direct bilirubin. These parameters can be used to assess liver function and/or damage. Transaminases are associated with hepatocellular

damage. Transaminases and bilirubins are involved and are more sensitive parameters of events related to liver function. [28] The extract is thought to have hepatoprotective effects due to the content of phenolic compounds including phenols, tannins, triterpenes, and alkaloids. [26,29] Analysis of the main kidney markers, urea, and creatinine, showed no change at the different doses of the extract in either the satellite or test groups. Of these markers, serum urea is often considered to be a more reliable predictor of renal function than serum creatinine. [30] Renal function was not affected by the extract. Serum triglyceride and total cholesterol levels in the test groups weren't significantly different from those in the blank groups. These parameters are part of the cardiac risk factors. [31] Analysis of these results suggests that the extract does not present a cardiac risk factor at these different doses. The results of the hematological parameters show that the HEELA didn't affect the red blood cell, hematocrit, and hemoglobin levels of rats exposed to the HEELA compared with the control. A decrease in the levels of these hematological parameters could lead to anemia. [26] No changes were detected in white blood cell, neutrophil, lymphocyte, or platelet counts compared with the control. Similar results were obtained [32] at a treatment dose of 500 mg/kg bw. Platelets are sentinel cells that contribute significantly contribution to anti-infective immunity. [33] The extract has no anti-infectious immune effect. As for white blood cells, their increase could be explained by a general inflammatory state [34] due to the extract's toxicity. According to these results, the extract does not induce anemia or immunodeficiency syndrome. These results would justify the low toxicity of the various doses of *Lannea acida* in the medium term. Diabetes is a disease that requires continuous treatment for better control. This suggests that the use of *Lannea acida* bark as an anti-diabetic treatment could be chronic. It is therefore necessary to assess the effect of *Lannea acida* trunk bark hydroethanol extract over the long term (sub-chronic toxicity). The residual moisture content of the plant material was 6.28% ± 0.05. This value is lower than 10%, which means that the plant material has been well dehydrated. [35] The yield of the hydroethanolic extract was 19.27% ±0.41 relative to the dry mass. This yield is slightly higher than that obtained [36] which was 18.26% in the methanolic extract. The results of the characterization tests showed that HEELA contains tannins, phenols, coumarins, alkaloids, triterpenes, and steroids. Previous studies on the phytochemical screening and pharmacological properties of *Lannea acida* have revealed the compound's content in aqueous, methanolic, ethanolic as well as petroleum ether ethyl acetate extracts of *Lannea acida* trunk bark. [3,11,37] Qualitative analysis doesn't reveal any flavonoids in the extract. Similar results were obtained [7] on the aqueous bark extract from the trunk of *Lannea acida*. On the other hand, the results obtained [10,12] show the content of flavonoids in the HEELA. The presence of coumarins in the extract was not reported by previous studies [13,38] Coumarins have also been isolated from other species such as *Lannea welwitschia*. [39] The polyphenol content of the extract, which was 649.77±0.36 mg EAG/g is lower than that obtained [14] which was around 786.75 ± 82.33 mg EQ/g for the ethanolic extract of *Lannea acida* trunk bark [10] found a level of 40.55±0.26 g EAG/100g for hydro-ethanolic extracts of *Lannea acida* bark. Our results on total flavonoid content are in line with those reported [10] which were of the order of 8.70±0.02 g EQ/100g. However, Compaoré et al [3] obtained a level of around 10.99 ± 0.12 mg EQ/100g, which is higher than our results. A study carried out in Cameroon revealed a flavonoid content of around 250.61 ± 48.17 mg EQ/g. [14] As for the total tannin content (79.71 ± 2.78 mg EAT/g), it is higher than that [of 32], which was 3.57mg EAT /100g. In Burkina Faso, a study by Compaoré et al [3] showed that the aqueous extract of *Lannea acida* trunk bark contained 21.57 ± 1.97 and 75.29 ± 4.68 mg ETA/100g of hydrolyzable and condensed tannins, respectively. The methanolic extract contained 17.92 ± 1.25 and 543.94 ± 18.67 mg ETA /100 g hydrolyzable and condensed tannins, respectively. The different variations observed during phytochemical screening and in the different rates in the quantitative analysis could be attributed to climatic and environmental factors such as geographical area, drought, soil, aggressions and diseases, genetic heritage including, harvest period and plant development stage, as well as the solvent used. [40] Secondary metabolites of plants are known for their beneficial effects and have been shown to have broad therapeutic potential in the treatment of chronic diseases including diabetes as well as

its complications. Phenolic compounds, phenols, flavonoids, and tannins have a diverse range of biological properties, including antioxidative, anti-aging, anti-inflammatory, and inhibitory properties. They also play an essential role in reproduction and growth. [41] According to Yuan et al [42], phenolic compounds have been shown to reduce the risk of developing or treating certain diseases such as diabetes. The presence of these compounds in the extract could explain its use in phytotherapy to treat pathologies including diabetes and infertility. [3,4]

CONCLUSION

Our study assessed the acute and subacute innocuity of the HEELA which it's used traditionally against diabetes. At a unique treatment dose of 2000 mg/kg bw, the hydroethanolic extract of *Lannea acida* trunk bark did not cause mortality after 14 days of observation. Similarly, medium-term use of the extract did not present any risk of toxicity, as no changes were observed in vital organs such as the liver and kidneys. In addition to hypoglycaemic and antihyperglycaemic effects, antioxidant activity, and anti-diabetic and reproductive effects will be investigated in the future.

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Ethics Statement

This research involved animal subjects, it was approved by Joseph KI-ZERBO University ethics committee CE-UJKZ/2023-14.

Informed Consent Statement

This research did not involve human participants, animal subjects, or any material that requires human ethical approval.

Clinical Trial Registration

This research does not involve any clinical trials.

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Not applicable.

Authors' Contribution

Abdelaziz Koussoube: Conceptualization, Methodology, Formal analysis, Data Curation, Writing- Original Draft; Filkpièrè Léonard Da: Supervision, Formal analysis, Data Curation; Basile Tindano: Methodology, Writing - Review & Editing; Téénganimbé Jean Luc Kabore: Investigation, Writing - Review & Editing; Elisabeth Ouedraogo: Investigation, Writing - Review & Editing; Balé Bayala: Conceptualization, Validation, Resources, Project administration, Funding acquisition.

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