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Hepatoprotective Activity of Aqueous and Ethanol Extracts from *Lophira Lanceolata* Van Tiegh. Ex Keay (Ochnaceae) Leaves on Wistar Rats Intoxicated with Carbon Tetrachloride

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ABSTRACT

The liver, through its multiple functions, is actively involved in the metabolic homeostasis of the body. However, it is not spared from various aggressions like other organs, which can lead to significant damage. Carbon tetrachloride is known in experimental models to be hepatotoxic, causing fibrosis, cirrhosis, and hepatocarcinoma. The aim of this study is to induce hepatotoxicity in Wistar rats and then treat them with both aqueous and ethanolic extracts of Lophira lanceolata leaves at different doses of 500 and 1000 mg/kg of body weight. Following the obtained results, the values of liver biochemical parameters indicating hepatic damage (AST, ALT, TB, CB, GGT, ALP) were significantly elevated 48 hours after the induction of hepatotoxicity but decreased significantly one week later compared to control rats. These biochemical results were confirmed by histological sections of the livers of intoxicated and treated rats. Phytochemical screening of the extracts revealed alkaloids, flavonoids, tannins, polyphenols, saponins, anthocyanins, quinonic derivatives, reducing compounds, and leuco-anthocyanins, and HPLC revealed many chemical compounds, especially flavonoids such as rhamnetin, flavone, rutin, quercetin, luteolin, flavone, which are believed to possess hepatoprotective properties.

Keywords: *Lophira lanceolate*; Aqueous and ethanolic extracts; Hepatoprotective

INTRODUCTION

Liver is the largest organ in the human body [1], and is essential for life due to its numerous functions. It has excretory, protective, and detoxifying functions in the body [2]. It is actively involved in metabolic homeostasis. Unfortunately, like any organ in the body, the liver is exposed to both exogenous and endogenous aggressions. Carbon tetrachloride (CCl_4) is a highly toxic chemical agent, the most famous one used to experimentally induce liver damage. Histopathological sections of liver tissues indicate that CCl_4 causes fibrosis, cirrhosis, and hepatocarcinoma [3]. According to the World Health Organization (WHO), 80% of the population resorts to traditional medicine [4] due to their attachment to ancestral culture and civilization for the treatment of diseases through herbal medicine, owing to the effectiveness, accessibility, and availability of medicinal plants [5].

In traditional medicine in Benin, traditional practitioners use the leaves of *Lophira lanceolata* to treat liver problems, according to our surveys. It is a plant widely distributed in Central and West African countries: Senegal, Cameroon, Sudan, Ivory Coast [6]. *Lophira lanceolata* belongs to the Ochnaceae family and grows in the dry areas of wooded savannah. The objective of this study is to induce hepatopathy in Wistar rats through carbon tetrachloride intoxication and then treat them with the administration of aqueous and ethanolic extracts of *Lophira lanceolata* leaves to provide scientific evidence for the use of these leaves by traditional practitioners in the traditional treatment of liver problems in Benin.

MATERIALS AND METHODS

Plant material

Leaves of *Lophira lanceolata* were collected from the Pahou forest (ALT: 10 m; N: 06°22.824'; E: 002°10.068; precision: 7 m) in the commune of Ouidah. The geographical coordinates of the plant collection site were recorded using a GARMIN GPS device during a hiking expedition in 2012, manufactured in America, under the ETREX version 3.51 software. Samples were identified at the National Herbarium of the University of Abomey-Calavi (UAC) and certified with the number AAC231/HNB. Subsequently, they were dried, ground, and stored in glass vials, protected from light and humidity, for the preparation of extracts.

Animal material

Female albino Wistar rats weighing between 120 and 150 g were used as the animal model and were provided by the Animal Facility of the Unit of Human Biology at the Faculty of Health Sciences in Cotonou. They were conditioned at a temperature of 25°C with a 12-hour light-dark cycle. Food and water were available 24 hours a day. *In vivo* studies were conducted in accordance with current guidelines for laboratory animal care and ethical directives for the investigation of experimental pain in conscious animals [7].

Chemical and pharmaceutical products

Ethanol 96°C and silymarin were used during the course of the present study.

Preparation of extracts

The aqueous extract was prepared by decoction of 50 g of powder in 500 mL of distilled water for 30 minutes. The resulting decoction was filtered through Whatman filter paper. The ethanolic extract was prepared by maceration of 50 g of powder in 500 mL with mechanical agitation for 72 hours. The macerate obtained was filtered through Whatman filter paper and then evaporated under reduced pressure at 50°C using a rotary evaporator (Büchi Rotavapor 200). The filtrate from the decoction and the evaporated macerate were subsequently lyophilized, scraped, and stored in the refrigerator.

***In vivo* experimental study of hepatoprotective effect**

The hepatoprotective effect of aqueous and ethanolic extracts of *Lophira lanceolata* leaves was investigated in Wistar rats using tetrachloride (CCl_4) induced hepatotoxicity. Female rats with a body weight ranging from 120 to 150 g were divided into seven groups (N=7) for our study, which consisted solely of curative interventions.

Control group (no intoxication, no treatment): Received nothing.

Positive control group (Intoxication without treatment: Received 0.5 mL of CCl_4/kg body weight, followed by 0.5 mL of distilled water daily for 7 days.

Reference group: Induced intoxication with CCl_4 (0.5 mL/kg) followed by treatment with silymarin at a dose of 70 mg/kg/day for 7 days.

Ethext 500 group: Induced intoxication with CCl_4 (0.5 mL/kg) followed by treatment with ethanolic extract of *Lophira lanceolata* leaves at a dose of 500 mg/kg/day for 7 days.

Ethext 1000 group: Induced intoxication with CCl_4 (0.5 mL/kg) followed by treatment with ethanolic Aqext of *Lophira lanceolata* leaves at a dose of 1000 mg/kg/day for 7 days.

Aqext 500 group: Induced intoxication with CCl_4 (0.5 mL/kg) followed by treatment with aqueous extract of *Lophira lanceolata* leaves at a dose of 500 mg/kg/day for 7 days.

Aqext 1000 group: Induced intoxication with CCl_4 (0.5 mL/kg) followed by treatment with aqueous extract of *Lophira lanceolata* leaves at a dose of 1000 mg/kg/day for 7 days.

Method of inducing hepatopathy

Wistar rats, fasting for 16 hours' prior, are subjected to acute toxic hepatitis induced by intraperitoneal administration of CCl_4. Intoxication is performed with a single dose of CCl_4, which induces significant necrosis in animals without causing mortality during the experiment. Several trials were conducted to determine the effective dose of CCl_4 and the timing of clinical manifestations. The effective dose is 0.5 mL of pure CCl_4 per kg of body weight, and the onset of toxic manifestations occurs within 48 hours. CCl_4 was dissolved in 50% extra virgin olive oil purchased from the market for experimental convenience, resulting in an intoxication solution of 1 mL/kg of rat body weight.

One hour after intoxication, the animals are exposed to water, and two hours later, they are presented with food.

Method of administration of extracts and silymarin

The various products are administered to the rats by gavage using a syringe equipped with a gavage cannula. Extracts from *Lophira lanceolata* leaves and silymarin are dissolved in 0.9% physiological saline. The administration of these products commenced 48 hours after the intoxications.

Study parameters

Clinical examination, assessment of mortality rate, kinetics of serum biochemical parameters, macroscopic examination of the liver, and histology were the selected parameters to evaluate the hepatoprotective activity of the two extracts from *Lophira lanceolata* leaves.

Clinical examination of animals

Throughout the experimental period, the animals underwent a daily clinical examination. Certain signs such as loss of appetite, refusal to drink, coloration of the ocular mucosa, appearance of feces, coloration of urine, and the behavior of the animals were considered to assess any potential toxic effects.

Evaluation of mortality rate

As CCl_4 is a highly potent hepatotoxic substance, the rats are therefore more susceptible to death. According to [8], the extent of the hepatoprotective activity of a substance can only be assessed by comparing the survival percentage produced by this substance in a test group with that of the control treated only with the hepatotoxic substance. We calculated, for each group, this rate expressed as a percentage relative to the group size using the following formula:

Mortality Rate = (n/N) x 100

(n=number of animals dead in the group; N=Group size)

Blood sampling

We performed three successive blood samplings on each group on the 1^{st} day (P0), the 3^{rd} day (P1), and the 10^{th} day (P2), 24 hours after the last treatment. The obtained blood samples were quickly centrifuged at 3500 revolutions per minute for 10 minutes. The supernatant was collected using a micropipette into Eppendorf tubes. All these tubes were stored in the freezer and transported to the Biochemistry Laboratory at CHD Abomey-Calavi.

Histological procedures

We conducted the histological study of the rat liver by sacrificing them 24 hours after the last treatment, which lasted for 7 days, on the eighth day. This histological study includes tissue fixation, processing, dehydration, clearing, impregnation, embedding, microtome sectioning, spreading, staining, microscope observation, and photomicrography. This process allows for a histological assessment of potential liver lesions. The manipulation protocol adheres to the one performed at the Laboratory of Animal Experimentation of the Unit of Human Biology

at the Faculty of Health Sciences located at ISBA.

The examination of the slides was carried out using a Motic photomicroscope equipped with a Motic Version 3.1 digital camera. The images were transferred after capture to an image processing software (Motic-Plus) provided with the photomicroscope. The images were captured at a magnification of (x400) and transferred in JPEG format to a USB drive.

Phytochemical screening

Phytochemical analyses, based on the differential reactions (coloration and precipitation) of the major groups of chemical compounds present in the aqueous and ethanolic extracts, were conducted following the method described by [7].

Identification by High-Performance Liquid Chromatography (HPLC)

HPLC analyses were carried out using products and solvents from Sigma Aldrich (France). This involved a series of commercially available standards used as reference compounds: Caffeic acid, ferulic acid, ellagic acid, syringic acid, tannic acid, gallic acid, chlorogenic acid, catechin, quercetin, luteolin, rutin, flavone, rhamnetin, isorhamnetin, chrysin, pyrogallol, and kaempferol. This analysis was conducted to highlight the presence of phenolic compounds, particularly flavonoids, in the ethanolic and aqueous extracts of *Lophira lanceolata* leaves. To achieve this, reference substances were utilized.

Identification of polyphenols within the different extracts was performed using HPLC-DAD HITACHI VWR 5430 analyses. These extracts were prepared in methanol (1 mg/mL). Detection was carried out using a C18 120 Å column (4.6 mm x 100 mm, 5 µ), AcclaimTM, through a binary program with systems consisting of solvent A (1% phosphoric acid solution at pH 3.78) as solvent A and solvent B (pure methanol). The solvent flow rate was 1 mL/min. The UV detector was set at a wavelength of 280 nm, and the injection volume was 10 µL. The column temperature was maintained at 25°C. The solvent rate programming used during the HPLC analysis is described in (Table 1).

Table 1: Table of solvent rate programming used during HPLC analysis.

Time (min)	0 to 20	20 to 25	25 to 30	30 to 35	35 to 45
Solvent A	80%-50%	50%-30%	30%-20%	20%-80%	80%
Solvent B	20%-50%	50%-70%	70%-80%	80%-20%	20%

RESULTS

Animal mortality

We recorded 5, 0, 1, 2, 1, and 1 death, respectively, in the untreated positive control (intoxicated), silymarin, Aqext 500, Aqext 1000, Ethext 500, and Ethext 1000 groups, corresponding to percentages of 83.33%, 0%, 16.66%, 16.66%, 33.33%, and

16.66%, respectively.

The extent of the hepatoprotective activity of a substance is measured by the mortality rate among animals in the test groups during the period of its administration, compared to the group of untreated intoxicated animals [8]. The recorded mortality percentages of 28.57%, 0%, 14.28%, 14.28%, 14.28%, and 14.28% in the untreated positive control (intoxicated), silymarin, Aqext 500, Aqext 1000, Ethext 500, and Ethext 1000 groups, respectively, indicate that CCl_4 is a potent hepatotoxic, and silymarin is highly hepatoprotective. The ethanolic and aqueous extracts of *Lophira lanceolata* leaves also appear to possess hepatoprotective properties. Thus, we investigated the hepatoprotective properties of our extracts by conducting an *in vivo* study on Wistar rats, initially examining the effect of the extracts on the Complete Blood Count (CBC) [9].

(Tables 2-4) present the values obtained for the CBC on the first day (P0) before CCl_4 intoxication, the third day (P1) after intoxication, and the tenth day (P2), 24 hours after the last treatment (Figures 1-3).

Table 2: Blood counts of normal-state rats (P0).

	Control control	**Positive control**	**REF**	**Aqext lot 500**	**Aqext lot 1000**	**Ethext lot 500**	**Ethext lot 1000**
RC	9.04 ± 0.54	9.05 ± 0.08	9.48 ± 0.39	8.32 ± 1.18	8.77 ± 0.22	8.74 ± 0.19	8.78 ± 0.17
Ht	51 ± 1	52.06 ± 0.15	55.53 ± 3.59	52.93 ± 4.95	49.8 ± 2.35	48.23 ± 2.92	49.63 ± 1.26
Hb	16.9 ± 1.1	16.7 ± 0.4	17.56 ± 1.11	17.1 ± 1.99	16.06 ± 0.51	16.00 ± 0.75	16.23 ± 0.40
MCV	56.5 ± 0.5	57 ± 1.3	58.6 ± 1.47	65.05 ± 15.68	56.83 ± 3.38	55.26 ± 2.57	56.5 ± 0.65
MCHCt	18.6 ± 0.6	18.4 ± 0.6	18.46 ± 0.50	21.06 ± 5.75	18.23 ± 0.55	18.26 ± 0.5	18.43 ± 0.35
MCHC	33.1 ± 0.9	32.06 ± 0.65	32.5 ± 1.38	32.21 ± 1.03	32.23 ± 1.07	33.1 ± 0.52	32.63 ± 0.23
WC	7.7 ± 1.7	11.5 ± 0.6	12.43 ± 6.58	17.56 ± 7.06	10.43 ± 1.53	12.86 ± 1.85	13.63 ± 3.49
LT	70 ± 1	73.5 ± 1.7	72.66 ± 12.66	69 ± 12.28	57.66 ± 19.08	74.66 ± 19.42	79.33 ± 8.32
Eosino	2 ± 0	3 ± 1	1.33 ± 1.15	0.66 ± 1.15	2.66 ± 2.30	0 ± 0	0.66 ± 1.15
Neutro	28 ± 1	23.5 ± 8.5	26 ± 11.78	30.33 ± 12.74	39.33 ± 17.92	25.33 ± 19.42	20 ± 7.21
PLT	530 ± 30	478 ± 128	576.66 ± 58.82	574.66 ± 15.27	552.33 ± 243.01	545 ± 17.43	613.333 ± 79.82

Note: RC: Red blood cells; Hematocrit: Ht; Hb: Hemoglobin; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration; WC: White Blood Cells; LT: Lymphocytes; MCHCt: Mean Corpuscular Hemoglobin Content; Eosino: Eosinophils; Neutro: Neutrophils, PLT: Blood Platelets.

Table 3: **Hematological parameter values for different batches of Wistar rats 48 hours after CCl_4 (P1) intoxication.**

	Control Control	**Positive control**	**REF**	**Lot Aqext 500**	**Lot Aqext 1000**	**Lot Ethext 500**	**Lot Ethext 1000**
RC	$4.92 \pm 0.92^{**}$	$5.77 \pm 0.64^{**}$	$6.14 \pm 0.15^{**}$	$5.92 \pm 0.42^{*}$	$6.44 \pm 0.74^{*}$	$7.2 \pm 0.2^{*}$	$6.62 \pm 0.62^{**}$
Ht	$29.6 \pm 0.4^{***}$	$34 \pm 3.4^{**}$	$36.03 \pm 1.81^{***}$	$33.25 \pm 1.85^{*}$	$40.7 \pm 0.9^{*}$	$40.65 \pm 0.25^{*}$	$38.65 \pm 3.55^{*}$
Hb	$9.4 \pm 1.1^{***}$	$10.8 \pm 1^{**}$	$11.43 \pm 0.47^{**}$	$10.85 \pm 0.85^{*}$	$12.6 \pm 0.4^{***}$	$13.4 \pm 0.1^{*}$	$15.85 \pm 2.15^{***}$

MCHCt	19.1 ± 0.4*	18.75 ± 0.35 NS	18.66 ± 0.55*	18.25 ± 0.15 NS	19.5 ± 1 NS	18.55 ± 0.65 NS	19.15 ± 0.35*
MCHC	31.7 ± 1 NS	31.75 ± 0.25 NS	31.86 ± 0.41 NS	32.55 ± 0.75 NS	30.9 ± 0.1 NS	32.95 ± 0.05 NS	33.1 ± 0.7 NS
WC	21.8 ± 1.2**	17.15 ± 4.05 NS	18.63 ± 4.55 NS	19.6 ± 1.1 NS	9 ± 1 NS	17.25 ± 1.75 NS	18.7 ± 2.7*
LT	56 ± 2***	41 ± 1**	45.33 ± 14.36 NS	59 ± 5 NS	46 ± 1 NS	29 ± 13*	37 ± 11*
Eosino	2 ± 2 NS	4 ± 2 NS	5.66 ± 3.78 NS	4 ± 2 NS	2 ± 0 NS	2 ± 2 NS	2 ± 0 NS
Neutro	42 ± 2***	51.5 ± 1.5*	47.66 ± 10.01NS	35 ± 3 NS	52 ± 1 NS	69 ± 11*	61 ± 11*
PLT	557 ± 18*	133.5 ± 6.5*	151.33 ± 134.50*	265.5 ± 105.5*	479 ± 79 NS	448.5 ± 79.5 NS	412 ± 103*

Note: *p<0.05; **p<0.01; ***p<0.001; NS non-significant difference from normal state at P0 stage.

Table 4: Blood counts of rats 24 hours after last treatment (P2).

	Control control	**Positive control**	**REF**	**Aqext lot 500**	**Aqext lot 1000**	**Ethext lot 500**	**Ethext lot 1000**
RC	7.14 ± 0.86 NS	8.25 ± 0.7	6.40 ± 2.58 NS	8.27 ± 0.45 NS	8.54 ± 0.36 NS	7.60 ± 1.67 NS	7.52 ± 0.83*
Ht	45.2 ± 1.2 NS	45.35 ± 2.85	37.4 ± 12.12 NS	44.8 ± 1.56 NS	47.3 ± 1.47 NS	45.06 ± 6.02 NS	46.5 ± 2.01 NS
Hb	14.4 ± 0.6 NS	15.25 ± 1.25	12 ± 4.51 NS	15.06 ± 0.41 NS	15.9 ± 0.60 NS	14.53 ± 2.37 NS	14.9 ± 0.26 NS
MCV	63.4 ± 1.2 NS	55.1 ± 1.2	59.93 ± 5.45 NS	54.26 ± 2.02 NS	55.43 ± 1.48 NS	60.06 ± 4.98 NS	62.8 ± 8.83 NS
MCHCt	20.1 ± 1.1 NS	18.45 ± 0.05	18.83 ± 0.60 NS	18.2 ± 0.79 NS	18.56 ± 0.66 NS	19.2 ± 1.04 NS	20 ± 2.34 NS
MCHC	31.8 ± 0.2*	33.55 ± 0.65	31.63 ± 1.86 NS	33.6 ± 0.3 NS	33.53 ± 0.40 NS	32.13 ± 1.17 NS	32 ± 0.85*
WC	11.9 ± 0.1 NS	14.35 ± 3.15	12.73 ± 7.29 NS	14.2 ± 3.40 NS	13.6 ± 1.77 NS	13.66 ± 1.76 NS	14.63 ± 6.99*
LT	70 ± 5***	34 ± 4	34.66 ± 4.16 NS	64.33 ± 9.01*	70.33 ± 2.08**	49 ± 14.17 NS	38 ± 10 NS
Eosino	0 ± 0 NS	2 ± 2	2 ± 2 NS	2.66 ± 0.57 NS	3 ± 0 NS	1.33 ± 1.15 NS	2 ± 2 NS
Neutro	30 ± 1**	64 ± 6	63.33 ± 5.77 NS	32.33 ± 7.50*	26 ± 2.64**	49.66 ± 13.05*	60 ± 11.13 NS
PLT	959 ± 11**	728.5 ± 34.5	676.66 ± 158.59 NS	701.66 ± 118.53 NS	783.66 ± 155.93 NS	829.33 ± 156.14 NS	769.66 ± 154.07 NS

Note: *p<0.05; **p<0.01; ***p<0.001; NS non-significant difference from normal state at P0 stage.

(Tables 5-7) show the values of biochemical parameters obtained on the first day (P0) before CCl_4 intoxication, on the third day (P1) after intoxication and on the tenth day (P2) 24 hours after the last treatment.

Table 5: Biochemical parameters recorded in the normal state of rats prior to CCl_4 (P0) intoxication.

	Control control	Positive control	Referencelot	Aqext lot 500	Aqext Lot 1000	Ethext lot 500	Ethext lot 1000
Creatinine	14.16 ± 0.52	15.25 ± 1.46	3.39 ± 2.20	6.11 ± 0.36	6.88 ± 1.41	6.15 ± 1.16	4.27 ± 2.47
AST	275 ± 45	150 ± 40	163.6 ± 31.85	206.82 ± 29.98	184.45 ± 21.74	185.77 ± 17.07	184.37 ± 42.91
ALT	72.38 ± 2.62	59.98 ± 17.56	45.68 ± 2.10	75.73 ± 13.61	52.95 ± 7.38	54.47 ± 12.94	44.65 ± 12.16
TB	5.58 ± 1.42	3.725 ± 0.40	6.35 ± 2.24	8.19 ± 1.06	4.90 ± 1.76	4.67 ± 2.62	5.2 ± 1.31
CB	0.15 ± 0.05	0.50 ± 0.03	0.6 ± 0.09	0.66 ± 0.11	0.42 ± 0.21	0.38 ± 0.10	0.35 ± 0.28
GGT	2.03 ± 0.53	3.61 ± 0.13	2.75 ± 0.83	2.76 ± 0.44	2.16 ± 0.70	1.84 ± 0.50	1.54 ± 0.37
ALP	531.85 ± 31.85	450.16 ± 44.21	341.63 ± 86.78	390.38 ± 16.98	263.16 ± 46.92	252.21 ± 31.40	253.55 ± 41.05

Table 6: Biochemical parameters recorded 48 h after CCl_4 intoxication (intoxicated state) (P1).

	Control control	Positive control	Reference lot	Aqext lot 500	Aqext lot 1000	Ethext lot 500	Ethext lot 1000
Creatinine	14.68 ± 5.37 NS	16.19 ± 3.34 NS	8.75 ± 1.05*	8.89 ± 0.71**	11.16 ± 2.60*	13.72 ± 3.76*	10.89 ± 3.01**
AST	261.73 ± 11.73 NS	266.595 ± 44.63***	292.53 ± 31.43*	271.19 ± 44.61*	299 ± 24.99*	277.06 ± 40.68*	236.01 ± 27.27**
ALT	126.43 ± 40.68 NS	105.94 ± 44.06*	128.69 ± 13.28**	124.7 ± 33.75*	152.35 ± 54.32*	142.35 ± 15.92**	137.09 ± 31.92*
TB	5.58 ± 1.58 NS	14.44 ± 5.64*	12.41 ± 1.22*	9.78 ± 0.36*	23.45 ± 0.55**	8.64 ± 0.65*	11.68 ± 2.78*
CB	1.48 ± 0.99 NS	5.285 ± 1.71*	5.68 ± 2.93*	2.63 ± 0.94*	8 ± 1**	0.77 ± 0.22*	7.69 ± 0.54*
GGT	3.43 ± 1.93 NS	31.43 ± 6.57**	25.24 ± 10.19*	26.81 ± 0.66***	29.42 ± 2.42***	4.98 ± 1.21*	16.06 ± 3.81*
ALP	1103.42 ± 474.51 NS	857.29 ± 90.19*	1163.86 ± 452.67*	1210.48 ± 308.52*	656.11 ± 98.59*	883.94 ± 111.85**	911.26 ± 71.64**

Note: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; NS non-significant difference from normal state at P0 stage.

Table 7: Biochemical parameters recorded 24 h after last treatment with various *Lophira lanceolata* extracts (P2).

	Control control	Positive control	Référence lot	Aqext lot 500	Aqext lot 1000	Ethext lot 500	Eth lot 1000
Creatinine	9.68 ± 6.38*	12.09 ± 7.34	5.55 ± 5.45*	3.82 ± 3.05*	4.06 ± 3.41*	3.19 ± 2.36*	3.68 ± 2.80*
AST	217.595 ± 12.98*	284.11 ± 33.81	231.21 ± 15.73*	249.70 ± 22.13*	177.42 ± 10.83**	133.6 ± 20.94*	179.30 ± 7.61*
ALT	124.1 ± 2.9***	163.65 ± 4.26	55.01 ± 10.38***	100.70 ± 34.48*	83.46 ± 37.33*	90.87 ± 11.75**	84.59 ± 38.86*
TB	5.58 ± 1.58*	15.91 ± 6.50	6.35 ± 2.24*	7.02 ± 1.76*	5.27 ± 1.29*	7.36 ± 1.70*	6.97 ± 3.45*
CB	0.5 ± 0.2*	7.36 ± 3.47	0.52 ± 0.36*	0.79 ± 0.01*	0.80 ± 0.09*	0.59 ± 0.07*	0.57 ± 0.12*
GGT	3.25 ± 1.66*	31.83 ± 5.59	4.96 ± 1.37*	3.32 ± 0.29**	3.12 ± 0.29**	2.65 ± 0.77**	5.17 ± 4.99**
ALP	1091.28 ± 91.28*	894.21 ± 24.30	689.19 ± 60.17*	596.75 ± 94.29*	529.70 ± 58.18**	533.64 ± 29.81**	336.20 ± 214.01*

Note: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; NS non-significant difference from normal state at P0 stage.

Histological sections of wistar rat liver subjected to carbon tetrachloride:

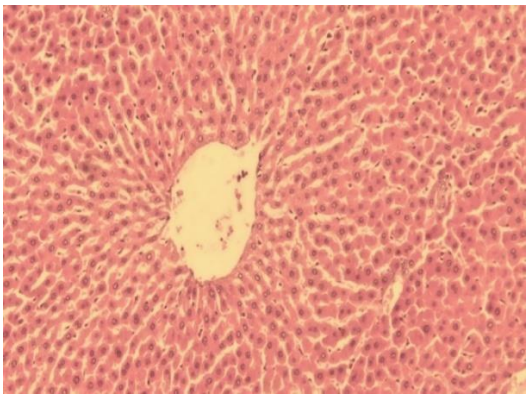

Figure 1A: Liver of control rat hepatic lobule showing hepatocytic trabeculae separated bysinusoidal capillaries and arranged around a centrilobular vein noted VC (HE x400).

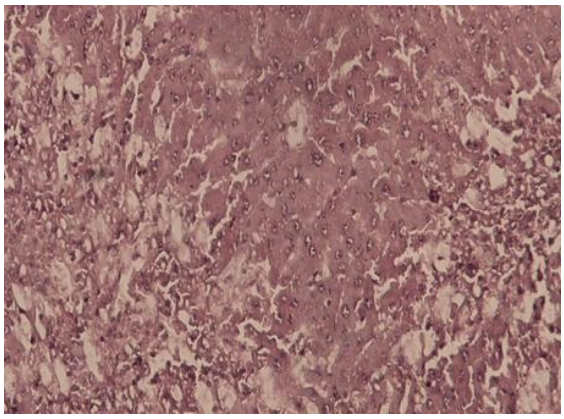

Figure 1B: Liver of rat intoxicated with CCl_4 hepatic lobule of rat treated with CCl_4 plus physiological water showing patchy necrosis of hepatocytes, particularly in the periphery of the lobule (HE x400).

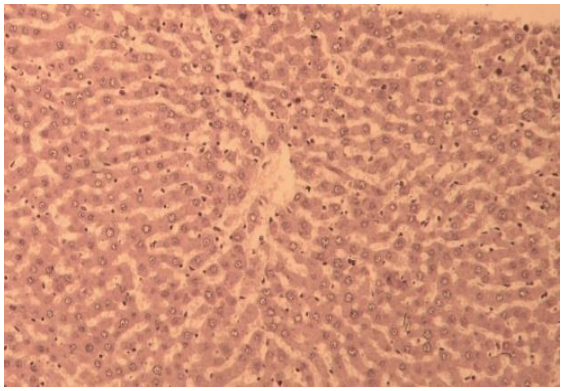

Figure 1C: Liver of rat treated with CCl_4 plus silymarin 70 mg/kg hepatic lobule of rat treated with CCl_4 plus silymarin 70 mg/kg body weight: Hepatic architecture relatively preserved (HE x400).

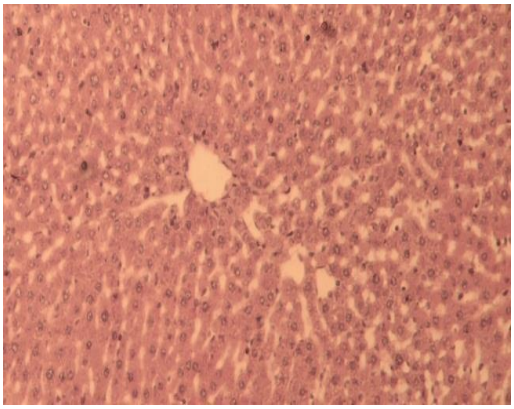

Figure 2A: Rat liver lobule treated with CCl_4 plus ethanolic extract of *L. lanceolata* at 500 mg/kg body weight: Although the lobular structure of the liver is preserved, a few hepatocytes with condensed nuclei persist (HE x400).

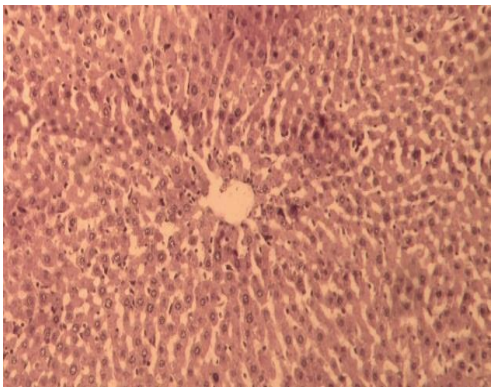

Figure 2B: Rat hepatic lobule treated with CCl_4 plus ethanolic extract of *L. lanceolata* at a dose of 1000 mg/kg body weight: No hepatocyte lesions were observed (HE x400), although the lobular structure of the liver was preserved, with the persistence of a few hepatocytes with condensed nuclei (HE x400).

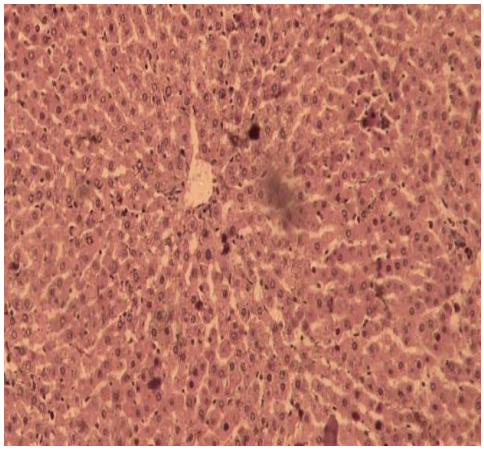

Figure 3A: **Rat hepatic lobule treated with CCl_4 plus aqueous extract of *L. lanceolata* at 500 mg/kg body weight: Although the lobular structure of the liver is preserved, a few hepatocytes with condensed nuclei persist (HE x400).**

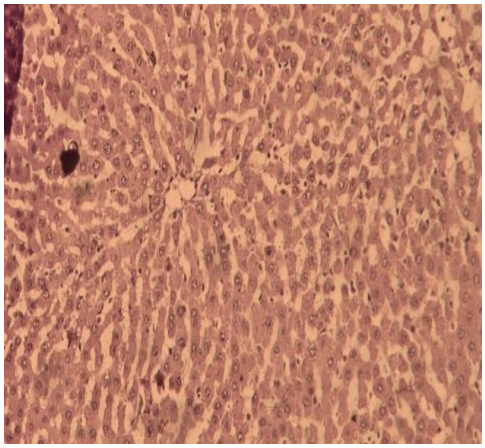

Figure 3B: Rat liver lobule treated with CCl_4 plus aqueous extract of *L. lanceolata* at 1000 mg/kg body weight: No hepatocyte damage observed (HE x400)

The chemical groups contained in both are given in Table 8 below.

Table 8: Chemical groups of *Lophira lanceolata* leaf extracts

Chemical groups	Aqueous extract	Ethanolic extract
Saponosides	+	+
Alkaloids	+	+
Polyphenols	+	+
Flavonoids	+	+
Tannins	+	+
Triterpenoids	-	-
Steroids	-	-
Cardenolides	-	-
Quinone derivatives	+	+
Anthocyanins	+	+
Leuco-anthocyanins	+	+
Reducing compounds	+	+
Anthracene derivatives	-	-
Mucilage compounds	+	-
Cyanogenic derivatives	-	-
Note: (+) = Presence; (-) = Absence.		

Phytochemical screening of *Lophira lanceolata* leaf extracts revealed alkaloids, flavonoids, tannins, polyphenols, saponosides, anthocyanins, quinone derivatives, reducing compounds and leuco-anthocyanins (Table 8). The greater efficacy of ethanolic and aqueous extracts of *Lophira lanceolata* leaves could be attributed to the chemical compound groups in these extracts, particularly to the presence of polyphenolic compounds in them. Among these compounds are

flavonoids, which are capable of inhibiting oxidants released by leukocytes and other phagocytes in the inflammatory zone, thereby maintaining inflammation [10-12].

Analysis of chemical composition by chromatographic profile of ethanolic and aqueous extracts of *Lophira lanceolata* leaves.

Chromatographic plots of *Lophira lanceolata* leaf extracts are shown in Figures 4 and 5.

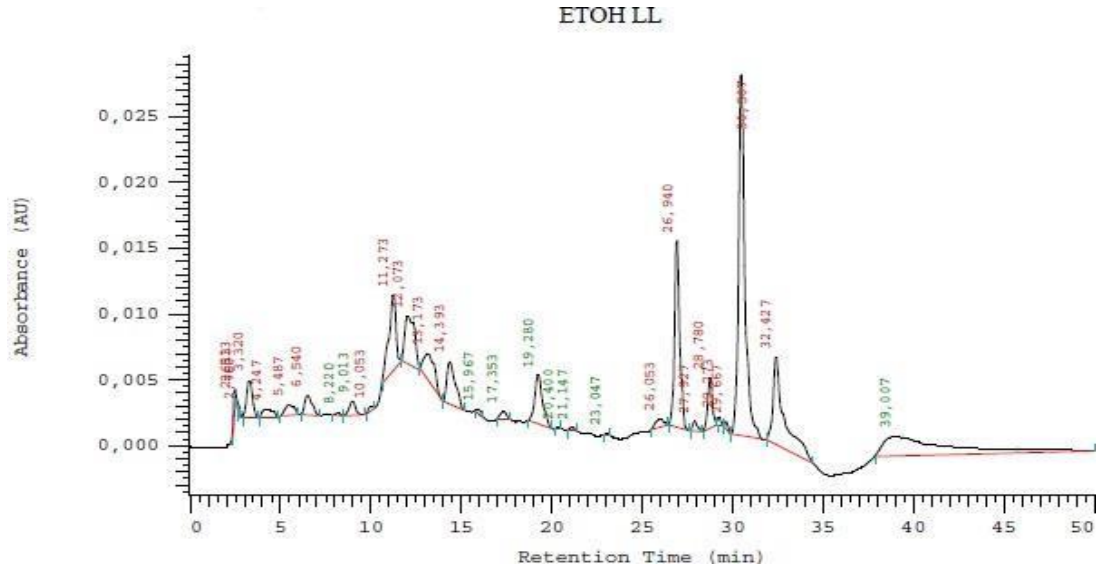

Figure 4: HPLC chromatogram obtained at 280 nm from the ethanolic extract of *Lophira lanceolata* leaves.
Note: Six (6) compounds identified: Tannic acid (Tr=2.51 min); ferulic acid (Tr=11.27 min); rutin (Tr=15, 95 min); quercetin (Tr=21.14 min); luteolin (Tr=28.78 min); flavone (Tr=8.62 min). The flavonoids present in the ethanolic extract of *Lophira lanceolata* are: Rutin, quercetin, luteolin, flavone.

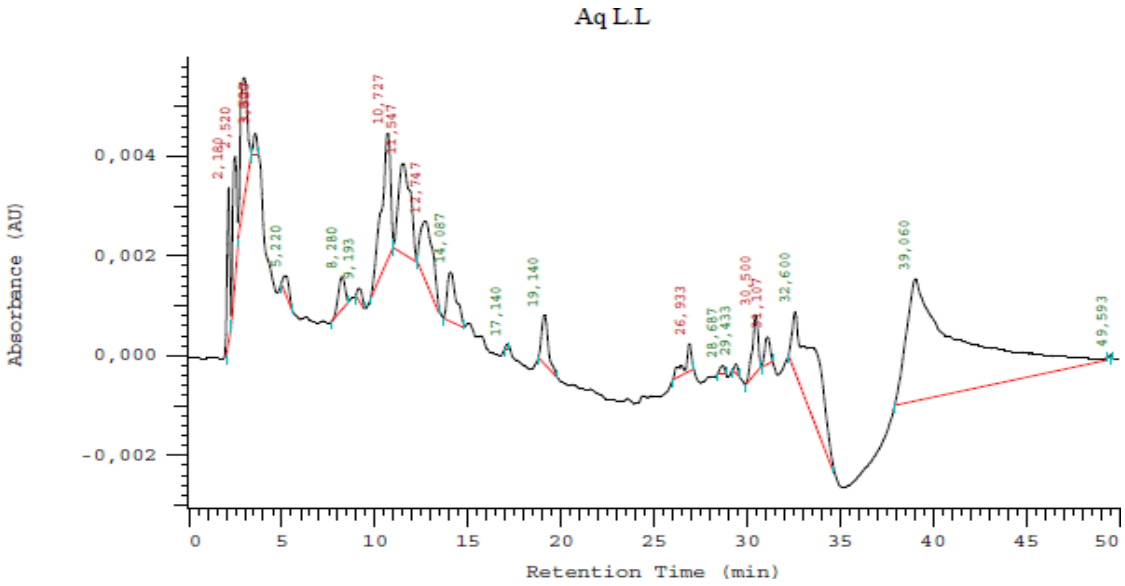

Figure 5: HPLC chromatogram obtained at 280 nm from the aqueous extract of *Lophira lanceolata* leaves.
Note: Six (6) composés identifiés: Acide tannique (Tr=2,52 min); catéchine (Tr=3,06 min); acide férulique (Tr=10,72 min); acide ellagique (Tr=17,14 min); rhamnétine (Tr=26,50 min); flavone (Tr=28,62 min). Les flavonoïdes présents dans l'extrait aqueux de *Lophira lanceolata* sont: Catéchine, rhamnétine, flavone**.**

Effects of various treatments on the blood count

For all our experiments, female Wistar rats weighing between 120 and 150 g were chosen as the animal model. Before exploring the various biochemical parameters, we examined the Blood Cell Count (CBC) of all rats in their normal state (P0), 48 hours after CCl_4 intoxication (P1) and 24 hours after the last treatment (P2). The results of the CBC can be found in the appendices (Tables 2-4). Analysis of these results showed that after 48 hours, CCl4 significantly altered haematological parameters ($p<0.05$), but these values were not significantly different 24 hours after the last treatment, except in the exteth 1000 batch, where we found that red blood cell, white blood cell and MCHC levels varied significantly compared with untreated intoxicated controls, but this variation could not account for the positive effect on CBC. The toxic effects of CCl_4 on CBC in rats were therefore not corrected by ethanolic and aqueous extracts of *Lophira lanceolata* leaves. Our study did not investigate the effect of extracts on CBC. We explored this avenue because in our literature searches, we found that some authors had explored this same avenue although their study focused on the hepatoprotective effect to see the effect of their extracts on CBC with very conclusive results.

Effects of *Lophira lanceolata* leaf extracts on biochemical parameters

With female Wistar rats chosen as the animal model, the degree of toxicity developed in these rats by the CCl_4 induction model and treatment after administration of ethanolic and aqueous extracts of *Lophira lanceolata* leaves is estimated by evaluation of serum biochemical parameters (AST, ALT, CB, TB, GGT, ALP) and by histological study of the liver [10]. The increase in the various biochemical parameters studied was not identical across all experimental batches. Instead, we noted a diversity in the sensitivity of rats to CCl_4. This may be due to the fact that rats have a very complex physiology. The creatinine assay enabled us to explore the consequences of intoxication on renal function in this study. CCl_4 is a nephrotoxicant, and the kidney's primary role is to eliminate waste from the body. Renal failure was observed in all batches intoxicated with CCl_4, which, on entering the body, undergoes a metabolic conversion reaction catalyzed by the microsomal cytochrome P450 enzyme system, giving rise to the more reactive free trichloromethyl radicals CCI_3•. These CCI_3• free radicals then bind to oxygen to give highly reactive CCl_3OO• trichloromethyl peroxide radicals, which are capable of reacting with liver macromolecules to trigger lesions or disturbances in liver function. Cytopathologically, liver damage is expressed by a hepatic cytolysis syndrome, the intensity of which can be easily monitored by the markers of this syndrome: AST and ALT transaminases. Together, these pathophysiological mechanisms explain the centrilobular hepatocyte necrosis and the significant increase ($p<0.05$) in transaminases observed in all intoxicated rats 48 hours after CCl_4 intoxication, with an AST/ALT ratio >1, signifying severe hepatitis. The same results were found by [11]. These biochemical parameters, which are the main markers of liver damage, fell significantly after one week's administration of the extracts, compared with the untreated intoxicated control batch. Similarly, all other biochemical parameters increased significantly 48 hours after CCl_4 intoxication. The significant increase in bilirubin and phosphatases is thought to be due to the disorganization of

hepatic architecture, affecting the permeability of pathways whose blockage is responsible for cholestasis. The increase in bilirubin could also suggest "free bilirubin jaundice", which would be due to excessive destruction of red blood cells (hemolysis), but in this case it's "conjugated bilirubin jaundice", rather linked to a biliary or hepatic disease, since the CBC, which is modified in rats 48 hours after CCl_4 administration, has not been corrected. This allows us to say that the ethanolic and aqueous extracts of *Lophira lanceolata* leaves corrected these values, which are the toxic effects of CCl_4 on the liver. This means that the altered liver has finally recovered its previous functions. LAP levels, which were significantly elevated 48 hours after CCl_4 administration, are indeed of hepatic origin, and this increase is mainly in the context of intra or extra-hepatic cholestasis, also fell significantly compared to untreated intoxicated controls, thanks to the administration of extracts during treatment.

Elevated GGT levels in the blood indicate liver damage, but without specifying the type of damage (hepatitis, cirrhosis, biliary tract damage, etc.). Generally speaking, the more severe the liver damage, the higher the GGT levels. The observed gamma GT level (31.83 ± 5.59) is therefore due to severe liver damage, which was eventually corrected by the administration of ethanolic and aqueous extracts of *Lophira lanceolata* leaves. Comparison of the various test batches with silymarin reveals that for creatinine, total and conjugated bilirubin and Gamma glutamyl transferase parameters, there was no significant difference between the various test batches and silymarin. Ethanolic and aqueous extracts of *Lophira lanceolata* leaves at different doses would therefore have the same protective effect as silymarin on the liver for these parameters. For plasma alkaline phosphatase concentration, Aqext 1000 and Ethext 500 had a greater effect han silymarin, with mean values of 529.70 ± 58.18 U/L and 533.64 ± 29.81 U/L respectively, compared with 689.19 ± 60.17 U/L. For Aspartate amino transferase, apart from the Aqext 500 test batch where the difference was not significant, there was a significant difference between the mean values of the Aqext 1000 (177.42 ± 10.83 U/L), Ethext 500 (133.6 ± 20.94 U/L), Ethext 1000 (179.30 ± 7.61 U/L) and silymarin (231.21 ± 15.73 U/L) test batches. For Alanine amino transferase, there was a significant difference between the Aqext 500 and Ethext 500 batches, with mean values of 100.70 ± 34.48 U/L and 90.87 ± 11.75 U/L respectively, compared with 55.01 ± 10.38 U/L for silymarin, showing that silymarin is more protective than Aqext 500 and Exteth 500 for this parameter. From the above, it can be deduced that Ethext 1000 is more protective than the other doses because it is the only dose for which, for three biochemical parameters: AST, ALT and ALP, the mean values obtained are significantly different from those of silymarin.

HPLC analysis of both ethanolic and aqueous extracts shows that they each contain six compounds. The ethanolic extract contains: Tannic acid, ferulic acid, rutin, quercetin, luteolin and flavone. The aqueous extract, on the other hand, contains tannic acid, catechin, ferulic acid, ellagic acid, rhamnetin and flavone. The reference product used in our experiments was silymarin, a highly hepatoprotective flavonoid. The aim of HPLC is to verify the presence of flavonoids in ethanolic and aqueous extracts of *Lophira lanceolata* leaves. Analysis of the chromatograms revealed that the flavonoids present in the ethanolic extract were rutin, luteolin, quercetin and flavone. Those present in the aqueous extract are: Rhanetin, catechin and flavone. Some authors have demonstrated the *in vivo* and *in vitro* anticancer properties of some of these flavonoids. The presence of these flavonoids would therefore be at the root of

the hepatoprotective effect observed. The difference observed in the two extracts is due to the different classes of flavonoids present in the two extracts, as they do not act in the same way. For example, the presence of a C2-C3 double bond can confer on certain flavonoids an activity 3 to 10 times greater than that of their analogues which do not. As the two extracts, ethanolic and aqueous, do not contain the same types of flavonoids, this difference in activity is understandable. The presence of flavonoids justifies the hepatoprotective activity of both extracts. As the liver is a highly complex organ, the hepatoprotective effect of the extracts needs to be confirmed by histological study, as CCl_4 is a hepatotoxic agent known to produce a centrilobular pattern characteristic of degeneration and necrosis. From the analysis of the histological results, we can say that the comparison of the architecture of the control rats with the positive controls on the one hand, of the latter with the intoxicated and then treated rats on the other, and finally of the control rats with the intoxicated and then treated rats, hepatotoxicity has set in and the positive controls are still suffering, which is confirmed by the biochemical parameters measured. The return to normal liver architecture observed in the intoxicated batches treated with different doses of ethanolic and aqueous extracts, and confirmed by the biochemical parameters, shows that these extracts do indeed have a hepatoprotective effect, like silymarin, whose mechanisms of action in protecting hepatocytes against toxins are known and can be summarized in the following four points:

- CCI_3• and CCl_3OO• free radicals are captured by the flavonoids in the extracts, preventing lipid peroxidation;
- Flavonoids strengthen membrane resistance and reduce permeability. This prevents free radicals from penetrating cells.
- The nucleus is not affected, DNA is not modified and genetic information remains intact.
- Flavonoids prevent collagen deposition by inhibiting hepatocyte transformation [12].

CONCLUSION

At the end of our research, the evaluation of liver biochemical parameters at different stages of the experiment revealed a clear reduction in transaminase, bilirubin, alkaline phosphatase and gamma-glutamyl-transpeptidase values following the administration of ethanolic and aqueous extracts of *Lophiera lanceolata* leaves during carbon tetrachloride intoxication of Wistar rats. These extracts appear to contain compounds with hepatoprotective properties, as suggested by the chromatograms obtained by HPLC, highlighting the presence of flavonoids with anti-inflammatory and anticancer activities favorable to the hepatoprotective effect. Moreover, histological analysis of the liver showed significant correction of CCl_4-induced liver damage, with increased efficacy of the ethanolic extract at a dose of 1000 mg/kg body weight. Thus, this study confirms the justification for the traditional use of *Lophiera lanceolata* leaves in the treatment of liver disease, while highlighting the need for further, in-depth research.

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