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Biological effect of the total aqueous extract of the leaves of *Lophira lanceolata* Van Tiegh. ex-Keay after induction hepatitis by paracetamol overdose in rats

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Abstract

Background: This work consisted of evaluating the potential hepatoprotective effects of the total aqueous extract of *Lophira lanceolata* leaves in rats intoxicated with paracetamol.

Materials and Methods: Six groups of six rats received solution by gavage, for group 1: distilled water, for group 2: paracetamol, for group 3: paracetamol and vitamin C, for group 4: paracetamol and extract 100 mg/kg BW, for group 5: paracetamol and extract 200 mg/kg BW and for group 6: paracetamol and extract 400 mg/kg BW. The experiment lasted 30 days and samples were taken on the 2nd day, the 15th and the 30th day for biochemical assays. The rats were then sacrificed, the livers removed, observed and weighed.

Results: The extract caused a significant and dose-dependent inhibition of the action of paracetamol. At the maximum dose of 400 mg/kg BW, it reduced and stabilized the concentrations of transaminases (AST and ALT), bilirubin, alkaline phosphatase and glucose, which had been elevated by the action of paracetamol. The extract also decreased the weight of the liver, the volume of which had been increased by the overdose of paracetamol. The extract restored the physical characteristics of the liver (color, texture and consistency) which had been damaged by paracetamol intoxication.

Conclusion: This study showed that total aqueous extract of *Lophira lanceolata* leaves has hepatoprotective activity. This activity could be due to the chemical compounds contained in the leaves of this plant.

Keywords: *Lophira lanceolata*; Paracetamol; Hepatoprotective Activity; Rat

1. Introduction

The liver is one of the body's most essential organs. It is the site of drug metabolism and storage of nutrient reserves ¬ [1]. Damage to this organ causes inflammation, known as hepatitis. Hepatitis has now become a real threat to human health [2,3]. According to [3], hepatitis was the seventh leading cause of death worldwide in 2013. It kills 1.4 million people per year [4]. According to the World Health Organization, the African continent accounts for 26% of the global burden of disease due to hepatitis B and C, with 125,000 deaths [5]. Hepatitis is contracted by 5 to 10% of the African population [4]. Studies by [6] carried out in Bangui, showed that several 5-year-old children presented with hepatic encephalopathy after acute poisoning due to paracetamol. In Côte d'Ivoire, the prevalence of hepatitis C is 3.3% of the general population [7,8]. Also, according to [9], the prevalence of hepatitis B is 12% in Côte d'Ivoire. Despite these alarming figures, the management of hepatitis B and C remains very limited [10]. Hepatitis can be of viral or drug origin.

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The study by [11] showed a very increased self-medication with paracetamol in Côte d'Ivoire, very often leading to other health problems including hepatitis. Treating hepatitis requires significant financial resources and long hospital stays. Thus, to alleviate their conditions, many patients turn to herbal medicine. Herbal medicine is the practice of treating or preventing illnesses using medicinal plants that are non-toxic, taking into account precise doses. Many of these medicinal plants have yielded new active molecules effective against recurring pathologies thanks to the efforts of chemists, hence the acceptance of traditional medicine as a form of alternative health [12]. Plants have therefore become today, an inexhaustible source for treating pathologies.

Ivory Coast, thanks to the diversity of its flora, contains several thousand plant species [13]. Some of these plants are used in the treatment of hepatitis. This is the case for the seeds of *Lophira lanceolata*, which are constantly used by Ivorian populations, particularly those in the north and west, to treat hepatitis.

Our work is therefore part of the promotion of local flora, as part of hepatitis therapy using *Lophira lanceolata* seeds. The aim is to provide a scientific basis for the traditional use of this plant by evaluating the potential hepatoprotective properties of its leaves in rats suffering from hepatitis linked to paracetamol poisoning.

2. Material and Methods

2.1. Material

2.1.1. Plant Material

The plant material is the aqueous extract of *Lophira lanceolata* leaves. Species identification was performed at the Botanical and Floristic Center of the Félix Houphouët Boigny University of Abidjan-Cocody.

2.1.2. Animal Material

The rats used in these experiments were male and female albino rats of the *Rattus norvegicus* species and of the *Wistar strain.* These adult male and female rats were eight (8) weeks old, with a body mass between 171 and 213 g. These rats were acclimated to room temperature of 25±3°C for five days before the start of the experiment. These animals were fed FACI® pellets. The tap water used to water them in feeding bottles was constantly renewed. The plastic cages containing the animals were cleaned three times a week with the wood shavings renewed. The rats were subjected to 12 hours of light and 12 hours of darkness daily. These rats came from the animal facility of the Physiology Laboratory, Félix Houphouët Boigny University.

2.2. Methods

2.2.1. Extraction Protocol

One hundred grams (100 g) of *Lophira lanceolata* leaf powder are decocted in one liter of distilled water. After mixing under magnetic stirring, the decoction is filtered twice (using cotton wool and Wattmann paper). The filtrate is evaporated at 65°C using a rotary evaporator and dried at 45°C in an oven. After drying, 18.2 g of the total aqueous extract of *Lophira lanceolata* leaves are obtained. The method used is that of [14].

Studies of the effect of the total aqueous extract of *Lophira lanceolata* leaves on paracetamol-induced hepatotoxicity

2.2.2. Experimentation

Selection and preparation of experimental animals

The choice of both sexes during the experiments was part of the study's context in order to assess the effect of paracetamol on rats in general. The animals were weighed using a precision balance at fixed times before the start of the studies. The animals were marked on the tail to identify them from 1 to 6 within each group. This allowed them to be monitored during the experimental period. However, the marking was renewed every week to prevent them from fading and to avoid identification errors.

Paracetamol Hepatitis Induction Method

The method of [15] was used to induce hepatitis in rats. Thirty-six (36) rats were divided into six (6) groups of six (6) and the solutions were administered orally using a feeding tube:

- Group 1: The animals have access to water and food and receive no treatment,
- Group 2: The rats receive 2 mL of a 200 mg/kg BW paracetamol solution (Doliprane®) for two weeks,
- Group 3: The rats are given a 2 mL paracetamol (Doliprane®) 200 mg/kg BW solution daily for two weeks, followed by 1 mL of a 100 mg/kg BW vitamin C solution for two weeks,
- Group 4: The rats receive 2 mL of a 200 mg/kg BW paracetamol solution (Doliprane®) daily for two weeks, followed by 1 mL of a 100 mg/kg BW solution of the aqueous extract of *Lophira lanceolata* leaves for two weeks,
- Group 5: The rats receive 2 mL of a 200 mg/kg BW solution daily for two weeks, Paracetamol (Doliprane®) for two weeks, followed by one mL of a 200 mg/kg BW solution of *Lophira lanceolata* leaf aqueous extract for two weeks,
- Group 6: Rats received 2 mL of a 200 mg/kg BW solution of paracetamol (Doliprane®) daily for two weeks, followed by one mL of a 400 mg/kg BW solution of *Lophira lanceolata* leaf aqueous extract for two weeks.

Every two days, new solutions were prepared, taking into account the new weight acquired by animal per group, until the end of the experiment. The first blood samples were taken on day 2 (D2) in all groups before hepatitis induction. The second blood samples were taken on day 15 (D15) after hepatitis induction in groups 2, 3, 4, 5, and 6. The last samples were taken on day 30 (D30) after treatment with vitamin C and the total aqueous extract of *Lophira lanceolata* leaves in groups 3, 4, 5, and 6.

Blood Collection

At the end of treatment and after a 12-hour fast, the animals were anesthetized with ether. Blood was collected from the retro-orbital sinus using sterile syringes for biochemical parameter determination. Blood from each rat was collected immediately into dry collection tubes.

Biochemical Parameter Assays

These studies were conducted at the Elivan laboratory in Treichville (Abidjan, Ivory Coast). Biochemical parameter assays (transaminases, bilirubin, alkaline phosphatase, and glucose) were performed using the method of [16,17].

2.3. Animal Sacrifice and Organ Harvesting

After blood collection, the livers were immediately isolated and washed with 0.9% NaCl saline. The livers were then observed, photographed, and weighed.

2.3.1. Calculation of Relative Liver Weight

The liver was harvested, weighed, and photographed at the end of all treatments. The livers were preserved in 10% formalin. Relative liver weight (RW) is calculated relative to body weight using the formula used by [18]:

- RW = Liver weight (LW) / Body weight (BW) x 100
- RW = Relative weight, LW = Liver weight, WB = Animal weight

2.3.2. Macroscopic Examination

This examination is qualitative and is limited to the macroscopic observation of the external structure of the whole liver sample. It takes into account color, consistency, and texture.

- Color: This is an important parameter in the diagnosis of liver diseases. In a normal state, the liver is bright reddish-brown in color. A change in color indicates a pathological state of the liver.
- Consistency: The normal liver is firm and soft to the touch. An alteration in these aspects indicates intoxication.
- Texture: The normal liver has a smooth texture. A change in texture indicates a pathological state.

2.4. Statistical Analyses

Results are expressed as means ± SEM (Standard Error of Mean). The analysis of variance (ANOVA) test will be used to determine the statistical significance of the results, followed by comparing the means of the different batches using the student t-test ($p < 0.05$). Graphpad Prism 8 Demo and Excel software were used to perform these statistical tests.

3. Results

3.1. Biochemical Studies

3.1.1. Assays before Paracetamol Administration (Day 2)

Normal AST levels were observed between 41.8 ± 3.5 and 45.8 ± 2.3 IU/L. ALT levels were between 24.6 ± 2.7 and 27.6 ± 3.4 IU/L. Normal values for total bilirubin, alkaline phosphatase, and glucose are between 0.84 ± 0.3 and 0.92 ± 0.5 mg/mL, between 98.2 ± 4.6 and 117.2 ± 6 U/L, and between 0.59 ± 0.5 and 0.71 ± 0.2 g/L, respectively (Table I).

Table 1 Normal values of biochemical parameters on day 2

	Dosage				
Treatments	**AST (UI/L)**	**ALT (UI/L)**	**Bilirubin (mg/mL)**	**Alkaline Phosphatase (U/L)**	**Glucose (g/L)**
Distilled water	45.8 ± 2.3	25.3 ± 3.1	0.92 ± 0.5	98.2 ± 4.6	0.61 ± 0.3
Paracetamol	41.8 ± 3.5	24.6 ± 2.7	0.84 ± 0.3	117.2 ± 6	0.71 ± 0.2
Paracetamol + Vitamin C	44.2 ± 3.1	27.6 ± 3.4	0.91 ± 0.5	104.5 ± 6.1	0.61 ± 0.1
Paracetamol + extract 100 mg/kg BW	43.5 ± 2.5	26.4 ± 3.4	0.88 ± 0.6	99.2 ± 2.3	0.59 ± 0.5
Paracetamol + extract 200 mg/kg BW	45.1 ± 3.4	27.1 ± 3.5	0.87 ± 0.2	111 ± 5.4	0.7 ± 0.3
Paracetamol + extract 400 mg/kg BW	44.1 ± 2.4	25.6 ± 4	0.9 ± 0.4	98.7 ± 5.4	0.69 ± 0.2

3.2. Assays after Paracetamol Administration (Day 15)

Transaminase levels increased significantly after paracetamol administration. Maximum values of 72.4 ± 3.2 and 49.4 ± 3.4 IU/L were observed for AST and ALT, respectively. Bilirubin, alkaline phosphatase, and glucose levels also increased. Maximum values were 1.9 ± 0.8 mg/mL, 197 ± 7.1 U/L, and 1.7 ± 0.4 g/L for bilirubin, alkaline phosphatase, and glucose, respectively (Table II).

Table 2 Biochemical Parameter Values after Paracetamol Administration on Day 2

	Dosage				
Treatments	**AST (UI/L)**	**ALT (UI/L)**	**Bilirubin (mg/mL)**	**Alkaline Phosphatase (U/L)**	**Glucose (g/L)**
Distilled water	45.8 ± 2.3	25.3 ± 3.1	0.92 ± 0.5	98.2 ± 4.6	0.61 ± 0.3
Paracetamol	63.4 ± 3.5	45.4 ± 3.1	1.1 ± 0.4	181.5 ± 5	1.5 ± 0.5
Paracetamol + Vitamin C	71.6 ± 4.5	46.4 ± 3.5	1.5 ± 0.6	197 ± 7.1	1.7 ± 0.4
Paracetamol + extract 100 mg/kg BW	72.4 ± 3.2	47.1 ± 4.2	1.9 ± 0.8	189 ± 4.1	1.5 ± 0.4
Paracetamol + extract 200 mg/kg BW	68.4 ± 4.2	49.4 ± 3.4	1.8 ± 0.6	184 ± 6.4	1.6 ± 0.4
Paracetamol + extract 400 mg/kg BW	71.5 ± 3.4	48.4 ± 3	1.7 ± 0.5	187 ± 4.9	1.6 ± 0.5

3.3. Dosages after extract treatment (Day 30)

After extract treatment, a dose-dependent decrease in biochemical parameters was observed (Table III). The extract significantly reduced AST, ALT, bilirubin, alkaline phosphatase, and glucose levels at doses of 200 and 400 mg/kg BW. At the maximum dose of 400 mg/kg BW, levels returned to 44.1 ± 2.3 IU/L, 24.2 ± 3.2 IU/L, 0.6 ± 0.4 mg/mL, 96.5 ± 4.1 U/L, and 0.51 ± 0.3 mg/mL for AST, ALT, bilirubin, alkaline phosphatase, and glucose, respectively. The extract has the same kinetics of action as vitamin C.

Table 3 Levels of AST, ALT, birubilin, alkaline phosphatase, and glucose after treatment with the extract on day 30

	Dosage				
Treatments	**AST (UI/L)**	**ALT (UI/L)**	**Bilirubin (mg/mL)**	**Alkaline Phosphatase (U/L)**	**Glucose (g/L)**
Distilled water	45.8 ± 2.3	25.3 ± 3.1	0.92 ± 0.5	98.2 ± 4.6	0.61 ± 0.3
Paracetamol	63.4 ± 3.5	45.4 ± 3.1	1.1 ± 0.4	181.5 ± 5	1.5 ± 0.5
Paracetamol + Vitamin C	42.1 ± 4.2 *	23.4 ± 3.2*	0.51 ± 0.5*	96.1 ± 4.5*	0.4 ± 0.1*
Paracetamol + extract 100 mg/kg BW	46.4 ± 4.1	28.4 ± 2.4	0.69 ± 0.4	99.5 ± 5.2	0.62 ± 0.4
Paracetamol + extract 200 mg/kg BW	45.1 ± 4.3*	26.9 ± 3.1*	0.62 ± 0.5*	97.4 ± 7.2*	0.58 ± 0.2*
Paracetamol + extract 400 mg/kg BW	44.1 ± 2.3*	24.2 ± 3.2*	0.6 ± 0.4*	96.5 ± 4.1*	0.51 ± 0.3*

3.4. Study of the physical characteristics of livers

3.4.1. Study of relative weights

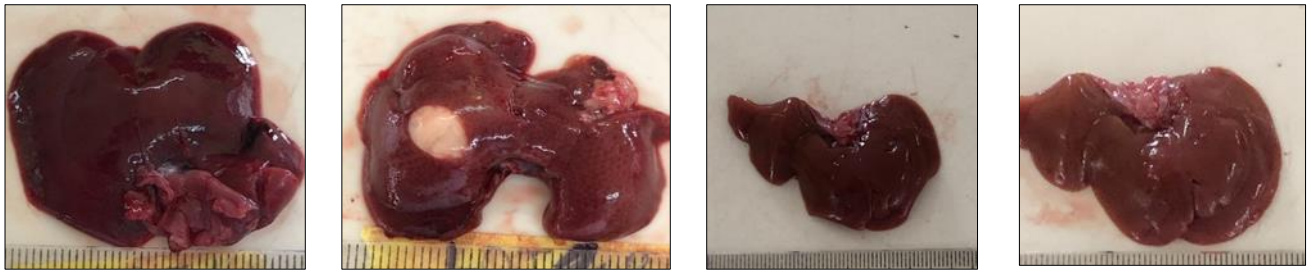
The relative weight in rats receiving only paracetamol was higher, at 7.2 ± 2.1%. This weight decreased dose-dependently in animals treated with the extract and those treated with vitamin C. The relative weight in rats treated with the extract at 400 mg/kg BW was 5.6 ± 2.2%, and that in rats treated with vitamin C was 5.2 ± 1.2% (see Table IV).

Table 4 Relative weights of the livers of different groups of rats

Distilled water	**Paracetamol**	**Paracetamol and vitamin C**	**Paracetamol and extract 100 mg/kg BW**	**Paracetamol and extract 200 mg/kg BW**	**Paracetamol and extract 400 mg/kg BW**
Poids relatif (%)	Poids relatif (%)	Poids relatif (%)	Poids relatif (%)	Poids relatif (%)	Poids relatif (%)
4.9 ± 1.2	7.2 ± 2.1	5.2 ± 1.2	6.8 ± 2.3	6.1 ± 3.1	5.6 ± 2.2

3.4.2. Macroscopic examination of the liver

This involved comparing the color, texture, and consistency of the livers of rats receiving only paracetamol and those of rats treated with vitamin C and the extract. Fat deposits were observed in the livers of rats treated only with paracetamol. In contrast, the livers treated with the aqueous extract and vitamin C gradually regained their color, texture, and consistency (Figure 1).



A - Normal liver	B - Liver affected by hepatitis	C - Liver treated with vitamin C	D - Liver treated with extract 400 mg/kg BW

Figure 1 Photos of the observed rat livers

4. Discussion

Liver intoxication due to paracetamol overdose is a commonly used method in experimental studies using animal models [16]. All of these studies refer to the measurement of biomarkers such as transaminases (ALT and AST), blood glucose, alkaline phosphatase, and bilirubin in the blood. According to [19], elevated blood concentrations of these parameters indicate liver intoxication.

The results of the initial assays showed normal or reference values in rats before administration of any substance. The studies conducted by [20] showed concentrations whose values are significantly close to those obtained in our studies. Paracetamol administration showed a clear increase in biochemical parameters such as transaminases, blood glucose, alkaline phosphatase and bilirubin. These results are consistent with those of the authors [16,21]. The mechanism of liver intoxication by paracetamol mainly targets parenchymal cells: this explains the increase in transaminases and blood glucose according to [22]. Paracetamol is metabolized by cytochrome P 450 to give an active metabolite N-Acetyl-P-benzoQuinone-Imine (NAPQI) leading to hepatic necrosis by peroxidation of membrane lipids (release of peroxyl radicals) which subsequently causes cell lysis [23]. Also, according to these latter authors, liver cells have glutathione for their defense, which constitutes their main antioxidant. Paracetamol overdose. Paracetamol poisoning causes glutathione depletion in the liver [24]. The active metabolite NAPQI produced by paracetamol overdose consumes glutathione, thus explaining the glutathione depletion in the liver, the immediate consequence of which is lipid peroxidation [25] and oxidation of protein thiol groups [26]. All these actions lead to an increase in transaminases, bilirubin, alkaline phosphatase and blood sugar according to [21]. Treatment with aqueous extract of *Lophira lanceolata* leaves at the maximum dose of 400 mg/kg BW and vitamin C significantly inhibited the effect of paracetamol on biochemical parameters compared to the control group. The concentrations of these biochemical parameters became normal and stable. These results are consistent with those of [21] with aqueous extract of *Pandanus odoratissimus* seeds and [27] with *Alchornea cordifolia* which showed a decrease in these parameters respectively with inhibition percentages ranging from 43 to 74%. Also the studies of [28] showed that the methanolic extract of *Gynandropsis gynandra* had hepatoprotective activity at the maximum dose of 400 mg/kg BW in rats with a significant decrease in biochemical parameters.

Relative weights were reduced after administration of the extract and vitamin C compared to the group of rats treated with paracetamol alone. These results are consistent with those of [20]. These data indicate that rats that received only paracetamol without treatment with vitamin C or the extract had increased liver weight due to induced hepatitis. Paracetamol overdose in rats showed significant variation in liver characteristics such as color, texture, and consistency. Fat deposition was observed on the liver of rats treated with paracetamol alone. The presence of fat on the liver is characteristic of hepatic steatosis. Our results are similar to those of [25,29] who demonstrated liver infection based on these observations. According to these same authors, these observed changes in characteristics are harmless and reversible. After administration of the extract, the liver practically regained its color, texture and consistency. These results are identical to those of [25] who showed the action of plant extracts on the macroscopic characteristics of the intoxicated liver.

The phytochemical screening of *Lophira lanceolata* leaves carried out by [30], showed the presence of flavonoids, anthraquinones, phenols, saponins, reducing sugars and tannins. The anti-hepatitis activity of flavonoids and phenols was shown in the studies of [21]. [16] also showed in their studies that saponins had anti-hepatitis activities. [31] showed a positive action of tannins on hepatitis. The chemical compounds present in *Lophira lanceolata* leaves could be responsible for the inhibition of the action of paracetamol on the liver.

5. Conclusion

The results of this study showed that *Lophira lanceolata* leaf extract has hepatoprotective activity. To this end, it significantly lowered the levels of liver biochemical biomarkers elevated by paracetamol overload in rats. The extract reduced the relative weight of livers affected by hepatitis and restored the liver's physical characteristics, including color, texture, and consistency. The hepatoprotective effect is believed to be due to the chemical compounds contained in *Lophira lanceolata* leaves.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there are no competing interests.

Statement of ethical approval

The equipment, handing and sacrificing of the animals were in accordance with the European Council legislation 87/609/EEC for the protection of experimental animals [32].

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