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Safety and *in Vivo* Anti-Inflammatory Activity of Ethanolic Extract of *Ficus umbellata* (Vahl.) Leaves

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Abstract

Toxicity is the totality of adverse effects, which can be functional and morphological lesions in a living organism, caused by a substance introduced in relatively high single doses or in small, repeated doses. The aim of this study was to assess the OECD-recommended acute oral toxicity and anti-inflammatory activity of ethanolic extract of *Ficus umbellata* leaves. Animals were given a single oral dose of 1000, 3000 and 5000 mg/Kg body weight (BW) of the extract. For the anti-inflammatory activity test, rats were given the ethanolic extract of *F. umbellata* leaves at doses of 100, 300 and 500 mg/Kg or aspirin® at a concentration of 100 mg/Kg PC orally, one hour before injection of 0.05 ml of 1% formalin under the plantar fascia of the rat's right hind paw. Paw volume measurements were taken one, two and three hours after formalin injection, using an electronic caliper. After 14 days of observation, no deaths were observed in treated rats. The LD50 of ethanolic extract of *Ficus umbellata* leaf powder is greater than 5000 mg/Kg body weight. This extract has no significant effects on hematological parameters and on the main markers of nephrotoxicity and hepatotoxicity for a single dose of less than 5000 mg/Kg PC. It reduces formalin-induced edema. Evaluation of the percentage inhibition showed that the extract had greater anti-inflammatory activity at 3

hours after the start of the experiment. However, better inhibition of inflammatory oedema of the paw of rats treated with 500 mg/Kg was observed at 5 hours after the start of the experiment, with a percentage inhibition of 69.23 ± 1.02, compared with the reference group treated with aspirin® 100 mg/Kg, which showed an inhibition of 63.50 ± 0.98. These results show that *F. umbellata* leaves possess anti-inflammatory activity, which would justify their use in traditional African medicine to prevent or treat inflammation.

Keywords

Ficus umbellata, Toxicity, Anti-Inflammatory, Edema, Inflammation

1. Introduction

Medicinal plants play a very important role in the fight against the majority of diseases in sub-Saharan Africa [1] and have therefore become indispensable in the treatment of many pathologies. In fact, they contain secondary metabolites (phytochemical compounds) that give them these properties [2]. In sub-Saharan Africa, medicinal plants are used to treat metabolic diseases such as hypertension, diabetes, etc. **[3]**. Several works have revealed that many herbal medicines are toxic and affect the health of users [4]. It is therefore important that the safety of plant extracts is further investigated, despite the fact that we have information on their pharmacological properties. Toxicity is the set of adverse effects that can be functional and morphological lesions in a living organism, caused by a substance introduced at a relatively high single dose or at small, long-repeated doses [5]. Several parameters are used to assess the toxicity of a substance. These include its mode of administration (oral, intravenous, intraperitoneal), the dose administered, the mortality rate observed, the histology of certain organs, weight trends, changes in certain biochemical blood parameters known as toxicity markers such as transaminases (ALT, AST), bilirubin, creatinine, urea [5].

Ficus umbellata is a plant that is widespread throughout Benin and whose leaves are used to treat several pathologies [6]. It is therefore necessary to assess the safety of this leaf in order to educate the population on the optimal doses not to be exceeded.

Inflammation is a reaction of the organism, implemented when the integrity of its morphological and biological constants is threatened [7]. Inflammation is caused by infections, physical and chemical agents. It can manifest itself through various symptoms such as edema (swelling or tumor), pain and heat or fever [8].

The aim of the present study was to evaluate the acute oral toxicity and anti-inflammatory activity of the ethanolic extract of *Ficus umbellata* leaf powder.

2. Materials and Methods

2.1. Animal Material

The animal material consisted of 120 - 180 g Wistar rats acquired from the la-

boratory. Pharmacology and Improved Traditional Medicines (LPMTA) from the Faculty of Science and Technology (FAST), University of Abomey Calavi (UAC) and with the Experimental and Clinical Biology Unit (UBEC), Biotechnology Research Laboratory Medical and Pharmaceutical (LaRBiMeP), National School of Biosciences and Biotechnology of Dassa-Zoumé (ENSBBA), National University of Science, Technology and Engineering of Abomey (UNSTIM) in Benin. They were fed pelleted feed and water.

2.2. Preparation of ethanolic extract

The collected sample was dried for three (3) weeks. It was then reduced to powder and stored in a suitable container for further handling. Extraction was dictated by bibliographical information on the chemistry of plant constituents. Ethanol was used as the solvent. Extraction took place in 3 stages: maceration, filtration and evaporation. 50 g of *F. umbellata* leaf powder is placed in 500 ml of ethanol. After maceration (for 72 hours), the product was filtered with filter paper, then with absorbent cotton placed in a funnel connected to a suction pump which accelerated filtration. After a few minutes, an exclusively liquid solution was obtained (the operation was repeated three times in succession). The filtrate obtained was placed in an oven at 45˚C to evaporate the ethanol. The dried extract was then scraped, weighed and the yield calculated.

2.3. Phytochemical Screening

Phytochemical screening was carried out according to the method used by Koutchiko [9].

2.4. Secondary Metabolite Assay

- **Determination of total polyphenols**

200 µl of extract was mixed with 1 ml of ten-fold diluted Folin-Ciocalteu reagent and 2 ml H_2O, and incubated at room temperature for 4 minutes. After adding 0.8 ml of 7.5% sodium bicarbonate to the mixture, total polyphenols were determined after 2 hours incubation at room temperature. Blue color absorbance was measured at λmax = 765 nm with a Shimadzu UV-VIS spectrophotometer. Quantification was performed using a standard curve for gallic acid at different concentrations (standard curve equation: y = 0.02x + 0.014 with R2 = 0.99). Results were expressed in milligrams of gallic acid equivalents (GAE) per g of dry extract.

- **Determination of total flavonoids**

1 ml of sample (prepared in methanol) was added to 1 ml of $AlCl_3$ solution (2% dissolved in methanol). After 10 minutes, absorbance was measured against the prepared reagent blank at λmax = 430 nm. Flavonoid concentrations were deduced from the calibration curve established with quercetin (0 - 35 µg/ml). Standard curve equation: y = 0.06x − 0.0002 with R2 = 0.98. Results were expressed in milligrams of quercetin equivalents per 1g of dry matter: mg EQ/1g of dry extract (mg EQ/g dry Ext).

- **Determination of condensed tannins**

Condensed tannins were determined using the vanillin method described by Julkumen-Titto. Vanillin reacts with free flavan-3-ols and the terminal units of proanthocyanidins, producing a red color whose intensity is proportional to the level of flavanols present in the medium, with an absorption maximum at 500 nm wavelength. Aliquots of 0.1 to 1 ml of the catechin stock solution (0.5 mg/ml) and extracts were introduced into a series of test tubes, the final volume in each tube being made up to 1 ml by addition of absolute methanol. 1.5 ml of 4% vanillin solubilized in methanol and 750 µl of 37% HCl (12M) were added at 1-minute intervals to each tube in the series and then placed in a water bath at 30˚C for 20 minutes. Results were expressed as milligrams of Catechin Equivalents (mg CE) per 1g of dry matter.

2.5. Acute *in-Vivo* Toxicity Tests

This study was conducted in accordance with OECD guideline 423 for the testing of chemicals [10]. At the start of the test, 8-week-old rats weighing between 120g and 180g were randomly selected. They were marked for individual identification and kept in their cages. Four (4) batches of three (3) rats were formed. One received only distilled water orally, while the other three received a single dose of 1000, 3000 and 5000mg/Kg B.W. respectively (as no information was available on the regulatory dose limit) of ethanolic extract of *F. umbellata* dissolved in physiological water. Animals were observed individually half an hour after administration of the extract, one hour later, and then daily. Samples were taken on D0 before and 14 days after administration of the extract. All observations were systematically recorded. Fourteen days later, the animals were sacrificed. Kidneys, liver and spleen were removed for histological study.

2.6. Parameters Assessed

- **Body weight**

Individual rat body weights were taken before extract administration, on D0 and then on D14. Weight variations were calculated and recorded.

- **Biochemical tests**

Biochemical parameters (bilirubin, AST, ALT, ALP, urea, creatinemia, uric acid) were determined using a Mindray-BS 240 automated system.

- **Hematological tests**

Hematological parameters were determined using a "SYSMEX XN-330" automated system. This machine counts white blood cells, red blood cells and platelets, and determines hemoglobin, hematocrit, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin Concentration and Average platelet Hemoglobin Content.

2.7. Histological Study of Harvested Organs (Kidneys, Liver and Spleen)

Kidneys, liver and spleen were removed, fixed in 10% buffered formalin and

embedded in kerosene. Sections of the samples (5 µm) were mounted on glass slides, deparaffinized and hydrated. For histological analysis, sections are stained with hematoxylin and eosin (H&E), following a standard protocol [11]. Photographs are taken at 400 X magnification.

2.8. *In Vivo* Anti-Inflammatory Activity Test

The study of the anti-inflammatory activity of the ethanolic extract of *F. umbellata* was conducted following the method described by Rahmani [12] and slightly improved. Rats were given the extract at doses of 100, 300 and 500 mg/Kg or aspirin at a concentration of 100 mg/Kg PC orally, one hour before injection of 0.05 mL of 1% formalin under the plantar fascia of the rat's right hind paw. Paw volume measurements were taken one, two and three hours after formalin injection, using an electronic caliper. Anti-inflammatory activity was assessed in terms of percentage reduction in edema in treated rats versus controls, using the following formula:

$$\text{Percentage } (\%) \text{ of inhibition} = \frac{(\%)\ \text{increase}_{\text{control}} - (\%)\ \text{increase}_{\text{treaty}}}{(\%)\ \text{increase}_{\text{control}}} \times 100$$

- % increase control: percentage (%) of average increase in circumference of edematous leg in control group.
- % increase treaty: percentage (%) of average increase in circumference of the edematized leg in the treatment group.

$$\%\ \text{increase} = \frac{V_t - V_0}{V_0} \times 100$$

V_0: volume of paw at t = 0 (before formalin injection).

V_t: paw volume at any time t.

2.9. Statistical Analysis

Data collected before and after treatment with the extract were tabulated and entered into Excel 2013. Normality and homogeneity of variances were checked with R Studio software using the Shapiro.test and Levene.test respectively; comparison of pre- and post-treatment data was carried out using the parametric paired two-sample test with R Studio software. Histograms showing the comparison of the mean rate of each parameter were produced using Graphpad Prism 9.5.1 (733) software. Significance is declared when the probability value P-value is less than 0.05.

3. Results

3.1. Extraction Yield

Extraction yield is calculated as the ratio between the mass of extract and the mass of leaf powder.

Yield = (Mass of extract/Mass of dry leaf powder) × 100

Yield = (30.55/150) × 100

The extraction yield was 20.37%. The extract has a black color.

3.2. Chemical Group Detection Test

Phytochemical screening enabled us to identify the various secondary metabolites contained in *F. umbellata* dried leaf powder. The qualitative results of this phytochemical screening test are summarized in Table 1.

3.3. Total polyphenol, flavonoid and tannin content

Total polyphenol, flavonoid and tannin contents are shown in Table 2. Total polyphenols are higher (125.65 ± 2.1 mgEAG/g dry extract) than flavonoids and tannins.

3.4. Clinical Signs Observed

The actions of the animals were carefully observed during the first 30 minutes after administration of the extract and throughout the 14 days of experimentation. It was noted that, apart from drowsiness, rats treated with 1000 and 3000 mg/Kg·BW showed no clinical signs, whereas those treated with 5000 mg/Kg·BW showed diarrhea, tremors and accelerated breathing. The results obtained are summarized in Table 3.

Table 1. Phytochemical screening (qualitative results).

Compound group	Metabolites	Characterization
Polyphenolic compounds	Flavonoids	+
	Gallic tannins	−
	Catechin tannins	+
	Anthocyanins	+
	Leuco-anthocyane	+
	Coumarin	−
Terpene compound	Steroids	+
	Triterpenoids	+
Nitrogen compound	Alkaloids	+
Heterosides	Saponosides	−
	Cyanogenic derivatives	−
	Reducing compounds	+
	Anthraquinones	+
	Mucilages	−

(+): Presence; (−): Absence.

Table 2. Secondary metabolite assays (quantitative results).

Metabolites	Total polyphenols (a)	Flavonoids (b)	Tannins (c)
Concentrations	125.65 ± 2.1 mgEAG/g of dry extract	28.80 ± 2.55 mgEQ/g of dry extract	20.98 ± 1 mgEC/g of dry extract

a: milligram of gallic acid per 1 gram of dry extract (mg EAG/g of dry extract); b: milligram of quercetin equivalents per 1 gram of dry extract (mgEQ/g of dry extract); c: milligram of Catechin Equivalents per 1 gram of dry extract (mgEC/g of dry extract).

Table 3. Clinical signs observed after extract administration.

Batch Signs	Control	1000 mg/Kg·BW	3000 mg/Kg·BW	5000 mg/Kg·BW
Drowsiness	−	+	+	+
Paralysis	−	−	−	−
Coma	−	−	−	−
Diarrhea	−	−	−	+
Lethargy	−	−	−	−
Tremor	−	−	−	+
Accelerated breathing	−	−	−	+

(+): Presence of sign; (−): Absence of sign.

3.5. Determination of LD50

After 14 days of observation, no deaths were observed, so the LD50 value for ethanolic extract of *Ficus umbellata* leaves is greater than 5000 mg/Kg body weight.

3.6. Variation in Animal Body Weight

Figure 1 shows the effect of the extract on animal body weight over the course of the experiment as a function of time. Analysis of the variation in body weight of animals given different oral doses of the extract showed a reduction in weight from Day 7 onwards. There was a downward trend in weight from Day 0 to Day 7 ($p > 0.05$). On Day 14, the decrease was very pronounced ($p < 0.0024$).

3.7. Effects of *F. umbellata* Extract on Haematological Parameters in Rats

Statistical analysis of the haemograms of the control and test batches showed that only blood platelets showed a significant difference from the control for doses of 3000 and 5000mg/Kg·BW ($p < 0.05$). The results obtained are summarized in Table 4.

Statistical analysis of the blood counts of the control and test batches showed that only blood platelets showed a significant difference from the control for the 3000 and 5000 mg/Kg·BW ($p < 0.05$).

3.8. Conjugated Bilirubin

Figure 2 shows the effect of *F. umbellata* ethanolic extract on conjugated bilirubin levels in rats. Conjugated bilirubin levels in the batches tested showed an upward trend at day 14. Analysis of variance results showed no significant difference whatever the dose administered ($p > 0.05$).

3.9. Effect of Ethanolic Extract of *F. umbellata* Leaves on Total Bilirubin Levels

Figure 3 shows the effect of ethanolic extract of *F. umbellata* on total bilirubin

Table 4. Effects of ethanolic extract of *F. umbellata* leaves on haematological parameters in rats.

Settings	Control	1000 mg/Kg·BW	3000 mg/Kg·BW	5000 mg/Kg·BW
White blood cells/(mm³)	8797 ± 703.28	8745 ± 573.51**a**	8387.66 ± 94.29**a**	8785 ± 215.54**a**
Hemoglobin (g/l)	15.86 ± 0.45	14.9 ± 0.3**a**	14.9 ± 0.21**a**	14.36 ± 0.12**a**
Red cells (10^6/mm³)	8.30 ± 0.65	8.50 ± 0.83**a**	9.75 ± 0.72**a**	9.78 ± 0.75**a**
Hematocrit (%)	41.05 ± 1.92	48.33 ± 3.05**a**	50.97 ± 3.48**a**	51.15 ± 3.1**a**
MCV (ftl)	50.58 ± 1.73	56.99 ± 2.18**a**	52.62 ± 7.34**a**	52.63 ± 6.92**a**
TCMH (Pg)	19.62 ± 1.93	17.62 ± 1.56**a**	15.33 ± 1.26**a**	14.75 ± 0.99**a**
MCHC (%)	38.53 ± 3.06	30.89 ± 1.61a	28.79 ± 0.96a	28.15 ± 1.97a
Platelets (g/l)	468.66 ± 49.86	411.66 ± 75.11**a**	311.33 ± 49.65**b**	297 ± 43.03**b**

A = statistically insignificant difference ($p > 0.05$); b = statistically significant difference ($p < 0.05$); MCHC: Mean Corpuscular Hemoglobin Concentration; VGM: Mean Corpuscular Volume; AHC: Average platelet Hemoglobin Content.

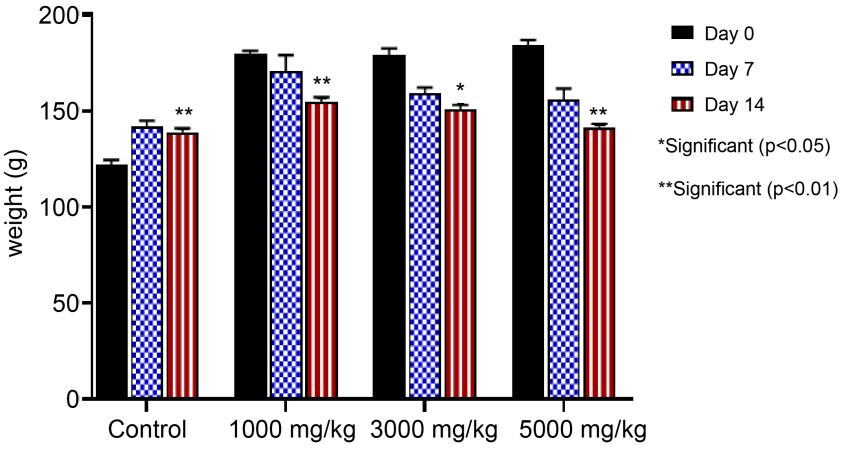

Figure 1. Effect of *F. umbellata* ethanolic extract on the average weight of Wistar rats.

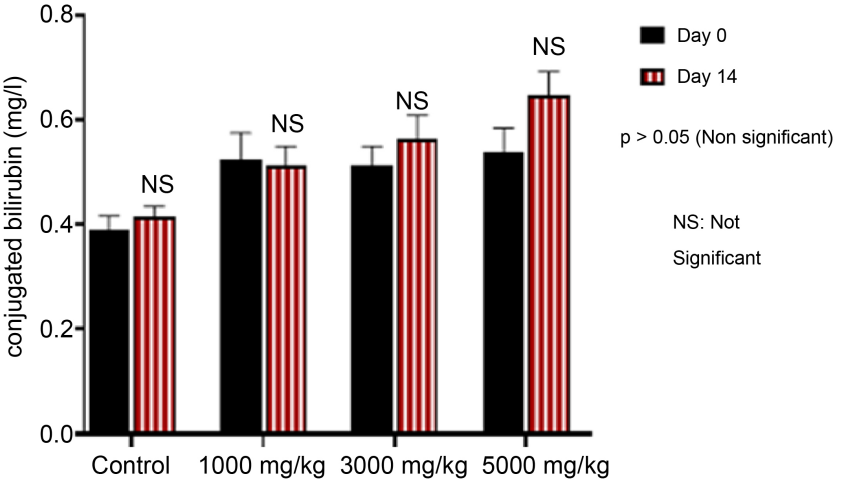

Figure 2. Effect of Ethanolic extract of *F. umbellata* on serum conjugated bilirubin levels in Wistar rats.

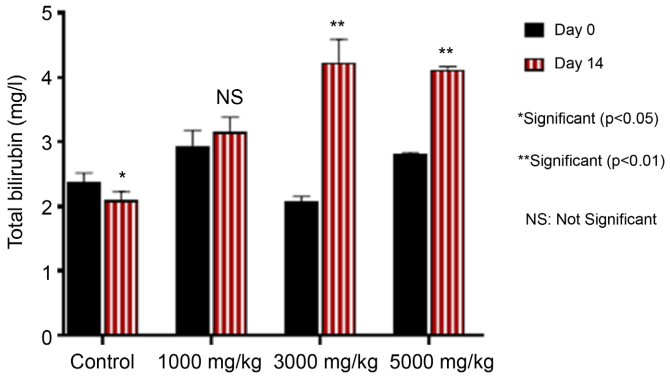

Figure 3. Effect of *F. umbellata* ethanolic extract on total bilirubin levels in Wistar rats.

levels in rats during experimentation. An increase in total bilirubin was observed in rat batches given 3000 and 5000 mg/Kg·BW with $p < 0.01$.

3.10. Effect of Ethanolic Extract of *F. umbellata* Leaves on Blood Creatinine Levels in Wistar Rats

Figure 4 shows the effect of ethanolic extract of *F. umbellata* on creatinine levels in rats during experimentation. Creatinine levels in the test batches showed no significant difference from the control ($p > 0.05$) on day 14.

3.11. Effect of Ethanolic Extract of *F. umbellata* Leaves on Uremia

Figure 5 shows the effect of ethanolic extract of *F. umbellata* on urea levels in rats. The urea levels of experimental batches given 5000 mg/Kg·BW increased with $p < 0.05$ after 14 days.

3.12. Effect of Ethanolic Extract of *F. umbellata* Leaves on uricemia in Rats

Figure 6 shows the effect of ethanolic extract of *F. umbellata* on uric acid levels in rats during experimentation. It shows that uric acid levels increased in batches of experimental animals given doses of 3000 mg/Kg·BW ($p < 0.05$) and 5000 mg/Kg·BW ($p < 0.01$) after 14 days. **Table 6** summarizes the effect of ethanolic extract of *F. umbellata* leaves on the various biochemical parameters assessed.

3.13. Effect of Ethanolic Extract of *F. umbellata* Leaves on Transaminase Levels

Figure 7 and **Figure 8** show the effect of ethanolic extract of *F. umbellata* on AST and ALT levels in rats during the course of the experiment. After 14 days, the transaminase values (ALT and AST) of the test batches only really increased with the 5000 mg/Kg·BW dose ($p < 0.05$), precisely at the ALT level.

3.14. Effect of Ethanolic Extract of *F. umbellata* Leaves on Alkaline Phosphatase Levels

Figure 9 shows the effect of *F. umbellata* ethanolic extract on alkaline phosphatase

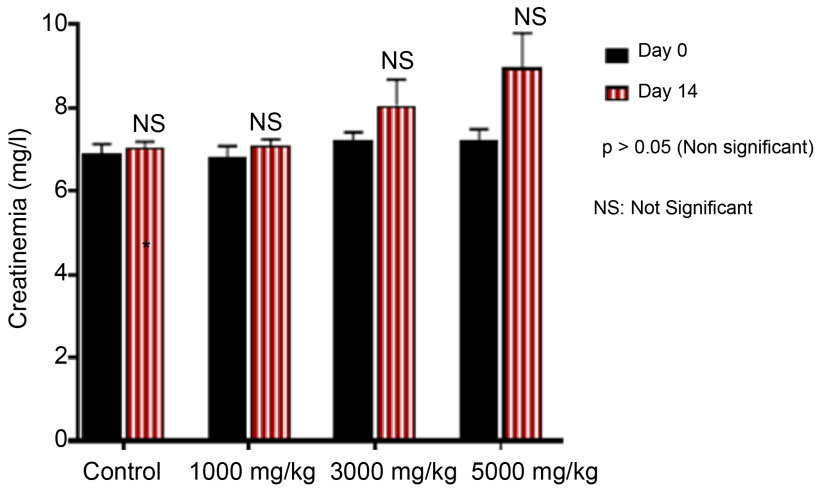

Figure 4. Effect of *F. umbellata* ethanolic extract on creatinine levels in rats.

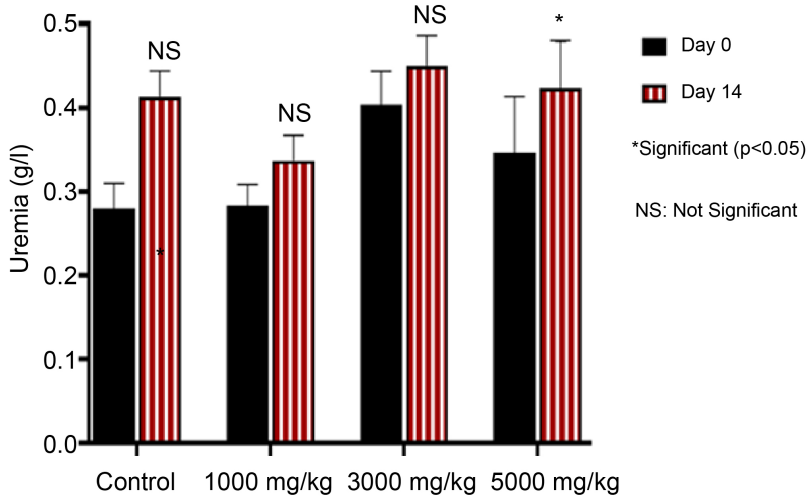

Figure 5. Effect of ethanolic extract of *F. umbellata* on urea levels in Wistar rats.

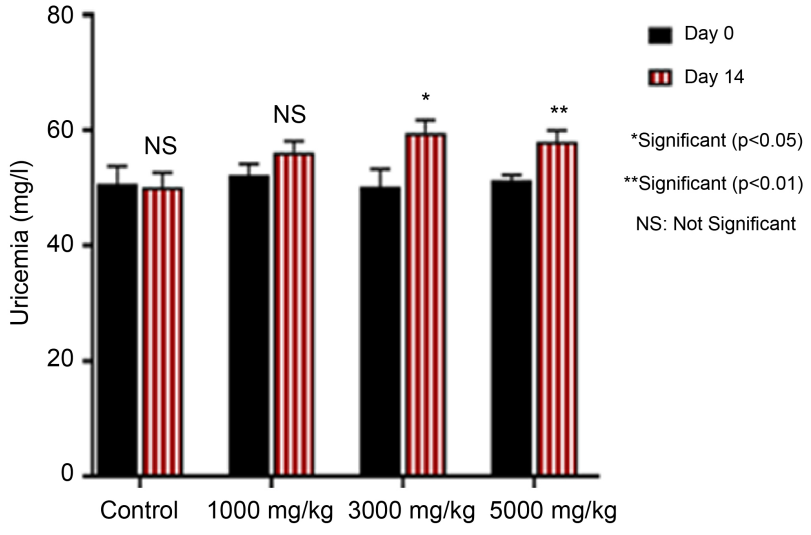

Figure 6. Effect of Ethanolic extract of *F. umbellata* on uricemia in Wistar rats.

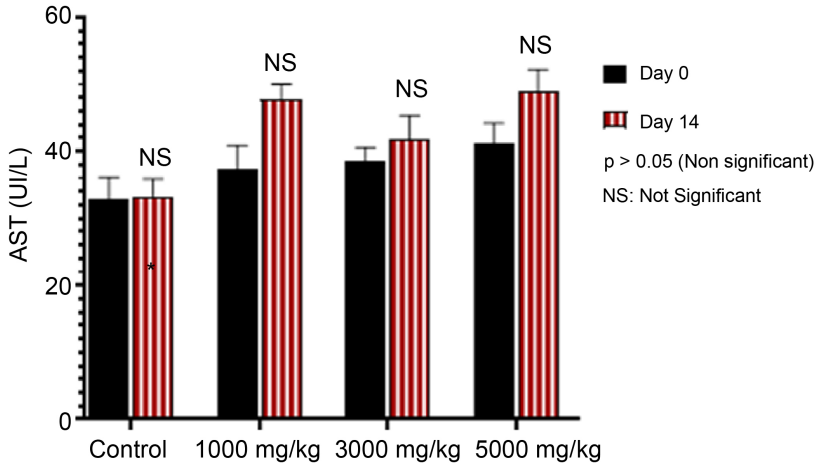

Figure 7. Effect of Ethanolic extract of *F. umbellata* on ASAT levels in Wistar rats.

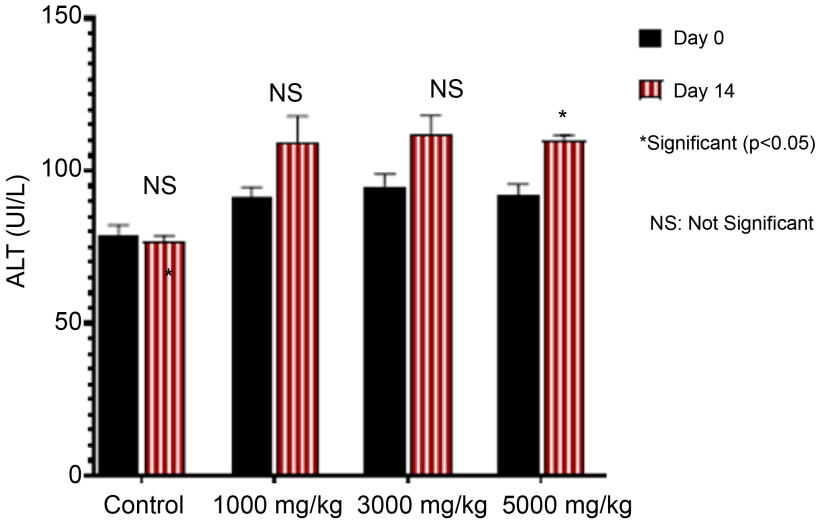

Figure 8. Effect of Ethanolic extract of *F. umbellata* on ALAT levels in Wistar rats.

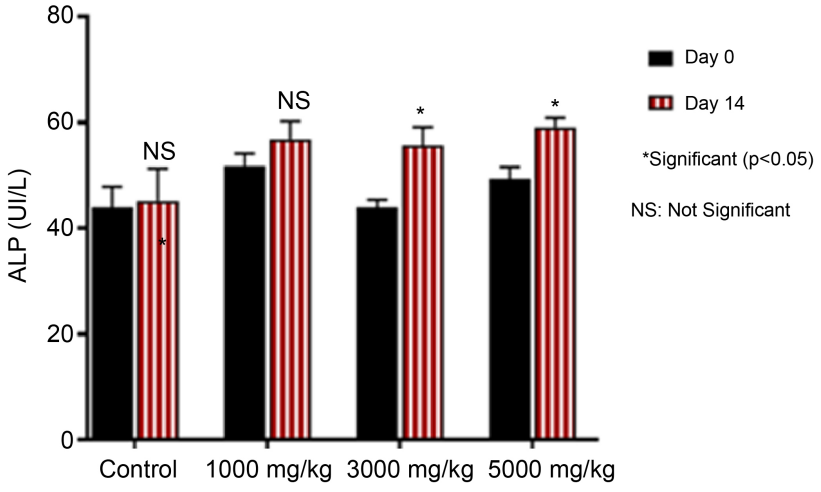

Figure 9. Effect of *F. umbellata* ethanolic extract on alkaline phosphatase levels in Wistar rats.

levels in rats during experimentation. Alkaline phosphatase levels in the test batches increased on day 14 ($p < 0.05$) with doses of 3000 and 5000 mg/Kg·BW.

3.15. Effect of Ethanolic Extract of *F. umbellata* Leaves on the Relative Weight of Harvested Organs

Ethanolic extract of *Ficus umbellata* had no significant effect on the weight of harvested organs. The results obtained are summarized in Table 5.

3.16. Histological Study of the Liver, Kidneys and Spleen in the Acute Toxicity Test of Rats Treated with Different Doses of the Extract

- **Liver histology**

Liver histology of rats treated with ethanolic extract of *Ficus umbellata* leaves was as follows:

The livers of rats treated with various doses of *Ficus umbellata* extract showed no visible atypia. Normal-looking hepatocytes (arrows) were arranged in radial cords around the centrilobular vein (CV). The venous sinusoids (S) are clearly

Table 5. Effect of ethanolic extract of *Ficus umbellata* on the relative weight of harvestedorgans after 14 days.

Weight (g)	Control	1000 mg/Kg	3000 mg/Kg	5000 mg/Kg
Liver	3.10 ± 0.08**NS**	3.05 ± 0.08 **NS**	3.03 ± 0.10 **NS**	3.01 ± 0.11 **NS**
Right kidney	0.30 ± 0.02 **NS**	0.31 ± 0.04 **NS**	0.30 ± 0.01 **NS**	0.30 ± 0.02 **NS**
Left kidney	0.31 ± 0.03 **NS**	0.31 ± 0.05 **NS**	0.31 ± 0.01 **NS**	0.30 ± 0.02 **NS**

NS: Not Significant.

Table 6. Effect of ethanolic extract of *Ficus umbellata* on the relative weight of harvestedorgans after 14 days.

Settings	Days	Control	1000 mg/Kg·BW	3000 mg/Kg·BW	5000 mg/Kg·BW
Conjugated bilirubin (mg/l)	**D0**	0.39 ± 0.02	0.52 ± 0.05	0.51 ± 0.03	0.53 ± 0.04
	D14	0.41 ± 0.02	0.51 ± 0.03	0.56 ± 0.04	0.64 ± 0.04
Total bilirubin (mg/l)	**D0**	2.37 ± 0.15	2.93 ± 0.25	2.07 ± 0.08	2.81 ± 0.02
	D14	2.09 ± 0.13	3.15 ± 0.22	4.23 ± 0.35**	4.12 ± 0.04**
Creat (mg/l)	**D0**	6.90 ± 0.22	6.80 ± 0.28	7.22 ± 0.18	7.22 ± 0.25
	D14	7.05 ± 0.13	7.09 ± 0.15	8.05 ± 0.62	8.97 ± 0.82
Uremia (g/l)	**D0**	0.28 ± 0.03	0.28 ± 0.02	0.40 ± 0.04	0.34 ± 0.06
	D14	0.41 ± 0.03	0.33 ± 0.03	0.45 ± 0.04	0.42 ± 0.06*
Uricemia (mg/l)	**D0**	50.74 ± 3.03	52.23 ± 1.9	50.22 ± 3.05	51.39 ± 0.83
	D14	50.11 ± 2.51	50.16 ± 1.93	59.54 ± 2.2*	58.04 ± 1.91**
AST (UI/L)	**D0**	32.77 ± 3.38	37.21 ± 3.67	38.54 ± 2.05	41.25 ± 2.98
	D14	33.3 ± 2.61	47.77 ± 2.25	41.82 ± 3.55	48.95 ± 3.25
ALT (UI/L)	**D0**	79.14 ± 3.17	91.36 ± 3.41	95.06 ± 3.95	91.95 ± 3.71
	D14	76.85 ± 2.02	109.26 ± 8.67	112.22 ± 5.98	110.05 ± 1.5*
ALP (UI/L)	**D0**	43.97 ± 3.84	51.68 ± 2.45	43.97 ± 1.38	49.34 ± 2.24
	D14	45.13 ± 6.13	56.85 ± 3.38	55.67 ± 3.472*	59.08 ± 1.76*

Data are presented as mean ± standard deviation; *: significant difference $p < 0.05$; **: significant difference at $p < 0.01$.

visible, as observed in control rats (Figure 10).

- **Renal histology**

Renal histology of rats subjected to ethanolic extract of *Ficus umbellata* leaves is as follows:

The renal parenchyma of rats treated with *Ficus umbellata* extract retained its typical appearance as observed in control rats. Glomeruli (G), proximal tubules (PT), distal tubules (DT) and collecting ducts (CC) showed no visible atypia (Figure 11).

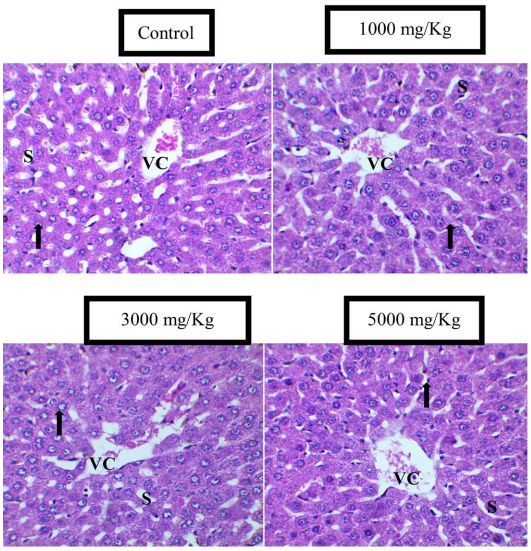

Figure 10. Liver histology of rats subjected to acute toxicity tests (400×).

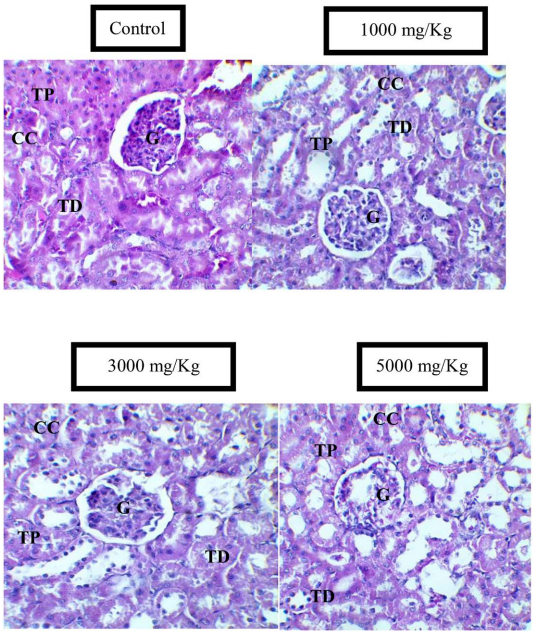

Figure 11. Renal histology of rats subjected to acute toxicity tests (400×).

- **Splenic histology**

Splenic histology of rats under the influence of ethanolic extract of *Ficus umbellata* leaves was as follows:

The splenic parenchyma was not activated in the acute oral toxicity test. As in control rats, the periarteriolar (MA) lymphocyte mantles around the central arteries (CA) and the germinal centers (GC) of the white pulp showed the typical appearance. The same applies to the sinusoids (S) and Billroth cords of the red pulp (Figure 12).

3.17. Anti-Inflammatory Activity

- **Percentage increase in hind leg oedema in rats treated with ethanolic extract of *F. umbellata* as a function of time**

Administration of ethanolic extract of *F. umbellata* at doses of 100 and 300 mg/Kg body weight (BW) significantly ($p < 0.05$) prevented 1% formalin-induced edema of the rat paw. The percentage increase in inflammatory paw oedema was 34.54 ± 2.73; 41.49 ± 5.56; 59.61 ± 1.81 for rats treated with 100 mg/Kg and 23.21 ± 2.13; 40.14 ± 2.27; 53.83 ± 4.04 for rats treated with 300 mg/Kg. compared with the control group treated with physiological water, whose edema increased by 41.31 ± 1.79; 75.24 ± 2.86 and 114.32 ± 6.86, respectively at 1, 3 and 5 hours after injection of 1% formalin. At 500 mg/Kg body weight, *F. umbellata* ethanolic extract showed better prevention of 1% formalin-induced paw edema than at 100 and 300 mg/Kg body weight. The percentages of increase in inflammatory paw edema were lower, at: 15.98 ± 1.62; 26.26 ± 1.42 and 35.19 ± 2.84, respectively at 1, 3 and 5 hours after injection of 1% formalin. Table 7 shows the percentage increase in hind leg oedema in rats as a function of time.

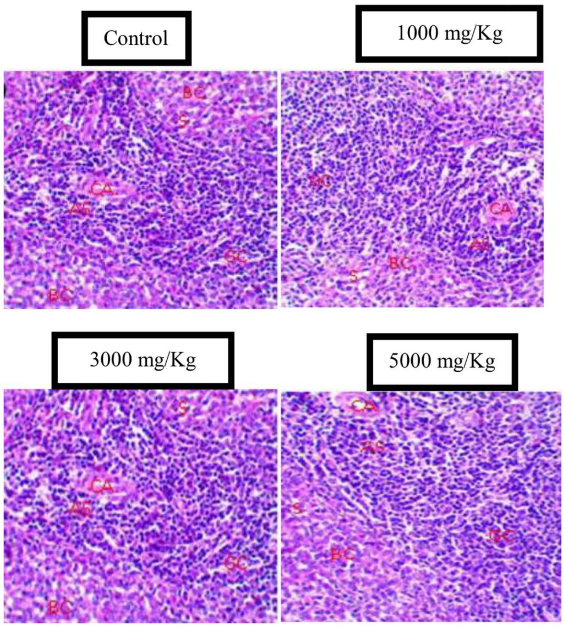

Figure 12. Splenic histology of rats in acute toxicity tests (400×).

Table 7. Percentage increase in rat hind paw oedema as a function of time.

Lots	Doses	Percentage (%) of increase in edema of the hind paw of rats as a function of time		
		1 h	3 h	5 h
Control	Physiological water	41.31 ± 1.79	75.24 ± 2.86	114.32 ± 6.86
F. umbellata	100 mg/Kg	34.54 ± 2.73	41.49 ± 5.56*	59.61 ± 1.81
F. umbellata	300 mg/Kg	23.21 ± 2.13	40.14 ± 2.27*	53.83 ± 4.04
F. umbellata	500mg/Kg	15.98 ± 1.62	26.26 ± 1.42*	35.19 ± 2.84*
Aspirin	100 mg/Kg	7.76 ± 0.55	23.55 ± 1.81*	41.58 ± 1.77*

Data are presented as mean ± standard deviation *: significant difference at $p < 0.05$.

Table 8. Percentage inhibition of rat hind leg oedema as a function of time.

Lots	Doses	Percentage (%) of inhibition of edema in the hind paw of rats as a function of time		
		1 h	3 h	5 h
F. umbellata	100 mg/Kg	16.46 ± 3.34	54.81 ± 9.98**	47.76 ± 2.63**
F. umbellata	300 mg/Kg	43.62 ± 7.29	46.63 ± 2.22*	51.67 ± 7.23*
F. umbellata	500mg/Kg	61.15 ± 5.5	65.01 ± 3.12*	69.23 ± 1.02**
Aspirin	100 mg/Kg	81.22 ± 0.84	68.69 ± 2.08*	63.58 ± 0.98**

Data are presented as mean ± standard deviation; *: significant difference at $p < 0.05$; **: significant difference at $p < 0.01$.

- **Percentage inhibition of hind paw oedema as a function of time in rats treated with Ethanolic extract of *F. umbellata***

Evaluation of the percentage inhibition showed that the extract had greater anti-inflammatory activity 3 hours later. However, a better inhibition of inflammatory paw edema at 500 mg/Kg·BW was observed at 5 hours with a percentage of 69.23 ± 1.02 compared to the reference group treated with aspirin 100 mg/Kg which showed an inhibition of 63.50 ± 0.98. Table 8 shows the percentage inhibition of hind-paw edema in rats as a function of time.

4. Discussion

Several works have revealed that to date many herbal medicines are toxic and affect the health of users [4]. It is important that the safety of herbal extracts be further investigated despite having information on their pharmacological properties. The total yield of ethanolic extraction of *F. umbellata* leaf powder was 20.37% ± 2.22%, with a black extract. Phytochemical analysis of the extract highlighted its richness in polyphenolic compounds, flavonoids, catechic tannins, leuco-anthocyanins, alkaloids, anthocyanins, anthraquinones, mucilages and sterols, as well as terpenes. Our results differ from those obtained by Yomakou [13]. In their work, we note the absence of anthocyanins, alkaloids, mucilages and sterols, as well as terpenes. This difference is linked to the plant's geograph-

ical distribution, the nature of the soil and the harvesting period. The ethanolic extract of *F. umbellata* leaves is very rich in polyphenols compared with flavonoids and tannins, which are not negligible. This result is in agreement with those of Yomakou [13] who demonstrated that the level of polyphenols in the ethanolic extract of *F. umbellata* leaves is higher than that of flavonoids and tannins. In the acute toxicity study, single-dose administration of the extract showed that (for the first 4 hours after administration), apart from drowsiness, rats treated with 1000 and 3000 mg/Kg·BW showed no clinical signs compared with the 5000 mg/Kg·BW dose, which caused diarrhoea, tremors and accelerated breathing. The LD50 was determined with the highest dose used (5000 mg/Kg), according to OECD guideline 423 [10]. After 14 days of observation, no deaths were observed in the treated rats. This suggests that the LD50 value for ethanolic extract of *Ficus umbellata* leaves is greater than 5000 mg/Kg. Whatever the dose administered, there was a significant reduction in rat body weight after 14 days. This may be due to a probable reduction in food consumption, but also to possible dose/absorption interactions and a reduction in the amount of food absorbed [14]. Our results corroborate those of Niamien [15], who had already shown a decrease in animal weight under the influence of ethanolic extract of *Ficus umbellata* leaves. Analysis of variance of the blood counts of control and treated rats showed that blood platelet levels were significantly reduced compared with the control ($p < 0.05$) after 14 days for doses of 3000 and 5000 mg/Kg·BW. The other hematological parameters showed no significant difference compared with the control batch. Blood platelets are cells that play a major role in primary hemostasis processes and play a key role in innate and adaptive immunity [16]. They are now recognized for their role in immunity, particularly antiviral immunity [17] [18]. With regard to the biochemical parameters explored, which provided information on the probable effects of the extract on the liver and kidneys, no significant differences were reported in rats given the 1000 m/Kg dose. However, doses of 3000 and 5000 mg/Kg·BW of the extract significantly increased bilirubin, uric acid and alkaline phosphatase levels. As for urea and ALT, the variation was only significant for the 5000 mg/Kg·BW dose. Transaminases are enzymes present in the liver, but also in muscle, kidney, pancreas and other tissues. They are synthesized in the cytoplasm of cells in these organs and discharged into the circulation, when these cells are damaged [19]. These enzymes increase in myopathy or myocardial infarction. ALT is more specific for liver damage, but AST is slightly more sensitive [20]. An increase in alkaline phosphatase (PAL) can have several causes such as liver disease, bone disease, or cancer [19]. Parameters such as uremia and uricemia are considered the main markers of nephrotoxicity [21]. In the case of this study, liver and kidney histology revealed no lesions. In general, the ethanolic extract of *Ficus umbellata* leaf powders has no significant effect on hematological parameters and the main markers of nephrotoxicity and hepatotoxicity for a single dose less than or equal to 5000 mg/Kg·BW. However, prolonged use of *Ficus umbellata* leaves at doses of 5000 mg/Kg·BW, could affect the liver and kidneys. In addition, anti-inflammatory tests indicate that this ex-

tract reduces formalin-induced oedema and has been shown to be effective at doses of 100, 300 and 500 mg/Kg, with similar activity to aspirin 100 mg. However, this anti-inflammatory effect is greater in the second phase (3 hours) of the inflammatory process. According to Sene [22], this may be due to mediators involved in the late phase of formalin-induced rat paw edema. According to Dosso [23], the reduction in edema could be explained by the inhibition of inflammatory mediators, notably histamine derived from the granulation of local basophils and mast cells, serotonins produced by the disintegration of blood platelets during the early hours of inflammation, and bradykinin released by a plasma protein system during the intervening hours.

5. Conclusion

Acute toxicity testing of ethanolic extract of *Ficus umbellata* leaf powders on rats showed no toxic effect. However, at doses greater than or equal to 5000 mg/Kg, prolonged use of these leaves could affect the liver and kidneys, since the levels of the main markers of nephrotoxicity and hepatotoxicity increase significantly above this dose. In addition, ethanolic extract of *Ficus umbellata* leaves proved effective in preventing inflammatory edema at doses of 100, 300 and 500 mg/Kg body weight.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Narcisse, B.H., Marie, G.N., Yves, T.O., Emilienne, P.Y., Legrand, N.N.B., Cynthia, A.E.D., *et al*. (2020) Pouvoir antibactérien des extraits aqueux et hydro-éthanolique du mélange d'écorces de tronc d'Albizia gummifera (J.F. Gmel.) C.A. Sm et Spathodea campanulata P. Beauv. *Journal of Applied Biosciences*, **154**, 15881-15887. https://doi.org/10.35759/JABs.154.5

[2] Victorin, H., Adjatin, A., Assogba, F., Gbénou, J. and Akoègninou, A. (2018) Etude Phytochimique et de cytotoxicité de quelques plantes utilisées dans le traitement de la stérilité féminine au sud-bénin. *European Scientific Journal*, **14**, 156-171. https://doi.org/10.19044/esj.2018.v14n6p156

[3] Mahamane, H., Diarra, M.L., Doumbia, S., Denou, A., Dembele, D., Diarra, B. and Sanogo, R. (2020) Plantes médicinales de l'Afrique de l'Ouest pour la prise en charge des affections respiratoires pouvant se manifester au cours de la Covid-19. *International Journal of Biological and Chemical Sciences*, **14**, 2941-2950. https://doi.org/10.4314/ijbcs.v14i8.22

[4] Aguinaga, Y.J., Claudiano, S.G., Marcusso, F.P., Ikefuti, C., Ortega, G.G., Eto, F.S., Cruz, C.D., *et al*. (2014) Acute Toxicity and Determination of Active Constituents of Aqueous Extract of *Uncaria tomentosa* Bark in *Hyphessobrycon eques*. *Journal of Toxicology*, **2014**, Article ID: 412437. http://dx.doi.org/10.1155/2014/412437

[5] Gisèle, E., Jacques, Y., Cécile, O.E., Vivien, M.B., Pascal, N.G., Emmanuel, M.M., *et al*. (2017) Étude de la toxicité aiguë et subaigüe de l'extrait au vin des graines de Carica papaya Linn. *Journal of Applied Biosciences*, **120**, 12077-12085.

[6] Lawin, I.F., *et al*. (2016) Vulnérabilité et stratégies endogènes de conservation des plantes utilisées dans le traitement du diabète dans les communes de Glazoué et Savè au Centre-Bénin. *International Journal of Biological and Chemical Sciences*, **10**, 1069-1085. https://doi.org/10.4314/ijbcs.v10i3.14

[7] Joachim, G.D., Judith, A.F., Pierre, L., Wilfrid, A.K.D.D., Richard, T., Pierre, D. and Mansourou, M. (2011) Propriétés anti-inflammatoires des extraits aqueux de Sterculia setigera Delile et du mélange Aframomum melegueta K. Schum-Citrus aurantifolia Christm et Panzer. *International Journal of Biological and Chemical Sciences*, **5**, 634-641. https://doi.org/10.4314/ijbcs.v5i2.72128

[8] Abena, A.A., Yayi, E., Gbenou, J.D., Moudachirou, M., Ongoka, R.P., Ouamba, J.M. and Silou, T. (2007) Comparative Chemical and Analgesic Properties of Essential Oils of *Cymbopogon nardus* (L) Rendle of Benin and Congo. *African Journal of Traditional, Complementary and Alternative Medicines*, **4**, 267-272. https://doi.org/10.4314/ajtcam.v4i3.31218

[9] Sègla, K.A.H., Eugène, A.S., Félix, G., Maximin, S., Madjid, A.A., Latifou, L., *et al*. (2022) Antioxidative Effects and Mechanisms of Antihypertensive Potential of *Croton gratissimus* Burch and *Schrankia leptocarpa* DC in Rats. *Annals of Hypertension*, **2**, Article No. 1011.

[10] OCDE (2001) Acute Oral Toxicity—Acute Class Method. OECD Test Guideline for Chemicals. No. 423.

[11] Senou, M., Khalifa, C., Thimmesch, M., Jouret, F., Devuyst, O., Col, V. and Gérard, A.-C. (2010) A Coherent Organization of Differentiation Proteins Is Required to Maintain an Appropriate Thyroid Function in the Pendred Thyroid. *The Journal of Clinical Endocrinology & Metabolism*, **95**, 4021-4030. https://doi.org/10.1210/jc.2010-0228

[12] Smahia, R., Nasser, B., Khaled, S. and Abdelkrim, C. (2016) Evaluation de l'activité anti-inflammatoire d'extraits aqueux de feuilles limoniastrum feei (plumbaginacea). *Algerian Journal of Arid Environment*, **6**, 80-86.

[13] René, Y.B.G.G., *et al*. (2021) Phytochemistry, Metabolites Quantification and Antioxidant Activity of *Calotropis procera* (Ait.) and *Ficus umbellata* (Vahl.), Plants Traditionally Used against Hemorrhoids in Benin. *International Journal of Current Research in Chemistry and Pharmaceutical Sciences*, **8**, 12-26.

[14] Kajimura, S. and Saito, M. (2014) A New Era in Brown Adipose Tissue Biology: Molecular Control of Brown Fat Development and Energy Homeostasis. *Annual Review of Physiology*, **76**, 225-249. https://doi.org/10.1146/annurev-physiol-021113-170252

[15] Magloire, N.C.J., Blaise, K., Inza, K. and Eliézer, N.K. (2018) Distribution spatio-temporelle de la pression du braconnage sur Eidolon helvum (Kerr, 1792), espèce proche de la menace (Côte d'Ivoire, Afrique de l'Ouest). *Journal of Applied Biosciences*, **126**, 12749-12757. https://doi.org/10.4314/jab.v126i1.12

[16] Nguyen, A.K.T. (2013) Inflammatory Role of Blood Platelets: Application in Transfusion. Hematology. Université Jean Monnet, Saint-Etienne, Tel-0105875.

[17] Flaujac, C., Boukour, S. and Cramer-Bordé, E. (2010) Platelets and Viruses: An Ambivalent Relationship. *Cellular and Molecular Life Sciences*, **67**, 545-556. https://doi.org/10.1007/s00018-009-0209-x

[18] Berthet, J. (2010) Functional Role of Toll-Like Receptor 4 Expressed by Blood Platelets as Inflammatory Immune Cells. Doctoral Dissertation, University Jean Monnet, Saint-Etienne, 121.

[19] Natacha, A., Melanie, A., Behanzin, J. and Alphonse, S. (2018) Evaluation of the

Toxicity of *Annona muricata* Leaf Extracts on Liver and Kidney Function and Investigation of Acute and Subacute Toxicity in Wistar Rats. *American Journal of PharmTech Research*, **8**, 189-217. https://doi.org/10.46624/ajptr.2018.v8.i1.013

[20] Bragança, D.S.A. (2017) Élévation des tests hépatiques. Service de médecine de premier recours, Département de médecine communautaire, de Premier recours et des urgencies. Hôpitaux universitaires, Genève, 13.

[21] Rodrigue, A., Loetitia, M., Justin, B., Elisabeth, H. and Alphonse, S. (2017) Impact des extraits éthyliques des feuilles de Hexalobus Monopétalus sur le métabolisme protéiques des rats Wistar. *International Journal of Multidisciplinary and Current Research*, **6**, 5. http://ijmcr.com/impact-des-extraits-ethyliques-des-feuilles-de-hexalobus-monopetalus-sur-le-metabolisme-proteiques-des-rats-wistar/

[22] Madièye, S., Mamadou, N., Sylva, B.F., William, D., Abdou, S., Awa, N.-S.Y., *et al*. (2016) Activité anti-inflammatoire de l'extrait aqueux des feuilles d'Elaeis guineensis Jacq. (ARECACEAE) sur l'œdème aigu de la patte de rat induit par la carraghènine. *International Journal of Biological and Chemical Sciences*, **10**, 2568-2574. https://doi.org/10.4314/ijbcs.v10i6.13

[23] Mamadou, D., Eugène, K.A., Doudjo, S., Ali, T. and Nafan, D. (2021) Activités analgésique, antiinflammatoire et antipyrétique d'un extrait aqueux des tourteaux de la pomme de cajou (*Anacardium occidentale* L.). *International Journal of Biological and Chemical Sciences*, **15**, 1842-1852. https://doi.org/10.4314/ijbcs.v15i5.12