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Assessment toxicological of *Opilia celtidifolia* leaves used in Traditional Medicine in Benin (West Africa)

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Abstract

Medicinal plants are continuously used as the first reflex of most populations in developing countries like Benin for primary health care. This unregulated or poorly rational use of these medicinal plants may present a potential toxicological risk. This study aimed to assess the toxicological characteristics of the ethanolic extract of the leaves of *Opilia celtidifolia* via the acute oral toxicity following the OECD 423 guideline of a single dose of 5000 mg/kg. Macroscopic observation of clinical signs of toxicity was recorded during the experimentation. Hematological and biochemical parameters of the liver and kidneys were evaluated with the histological study of these organs. As result, no mortality and no significant influence of the ethanolic extract of the leaves of *Opilia celtidifolia* on the biological parameters of the liver and the kidneys of the rats were not observed. These outputs reflect the safety of the leaves of *Opilia celtidifolia* and justify its use in traditional medicine in Benin (West Africa).

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Introduction

The use of medicinal plants for health care is an art practiced by a major part of the world's population from antiquity to the present day. This practice known as traditional medicine is an alternative to modern health care. It occupies a place of choice in the health care of populations, especially those in low-income countries (Agbodjento *et al.*, 2020). In 2002, WHO estimates that more than 80% of the world's population, for multiple reasons use this medicine for their primary health care (OMS, 2002). This medical practice that constitutes an ancestral heritage is based on the use of medicinal plants and other ingredients in the form of medicinal remedies (Bayaga *et al.*, 2017; Klotoé *et al.*, 2013). Thus, the population uses these medicinal recipes without having the guarantee of the safety of these medicinal plants. Data from the literature indicate that the use of medicinal plants may present a potential toxicological risk depending on the dose and duration of their use (Agbodjento *et al.*, 2020). In addition, for some authors, the mismatched combination of medicinal plants poses risks of toxicity (Zerbo *et al.*, 2007; Béné *et al.*, 2016). These observations complain of the need to undertake scientific studies aiming to assess the toxicological characteristics of these medicinal plants in order to secure their use in Phytotherapy widely practiced in developing countries such as Benin.

Africa has a rich flora of vegetable species widely used in traditional African medicine. Akoègninou *et al.* (2006) estimated the ethnobotanical potential of Benin to 2807 plant species. *Opilia celtidifolia* (*Syn Opilia amentacea* Roxb) is a medicinal plant of the Opiliaceae family belonging to the Beninese flora and which has many ethnopharmacological applications (Akoègninou *et al.*, 2006). In Benin, leaf and stem decoction is used against many diseases including malaria, epilepsy, asthenia, myalgia, headache and viral hepatitis (Akoègninou *et al.*, 2006; Guinnin *et al.,* 2015). In Togo, its same medicinal indications have been reported in the study of Adjanohoun *et al.* (1986). In the traditional medicine of Mali, the leaves and roots are used in the treatment of malaria, dermatitis and also to relieve joint pain and vomiting (Grønhaug *et al.*, 2008; Nordeng *et al.*, 2013). In Guinea Bissau, the leaves of the plant are used in the treatment of sexually transmitted diseases (Catarino *et al.*, 2016). In Ivory Coast, *Opilia celtidifolia* leaves are used in the traditional treatment of malaria and cough (Ambe and Malaisse, 2014). The above-mentioned evidence attests to the importance given to *Opilia celtidifolia* in traditional African medicine. However, very few scientific studies have been carrying out about the safety of the leaves of the plant.

The objective of this study was to produce data on the toxicity of the leaves of *Opilia celtidifolia* used in traditional medicine via acute oral toxicity.

Material and methods

Plant material

Fresh leaves of *Opilia celtidifolia* harvested in Abomey-Calavi town were used as plant material. This plant organ was certified at the National Herbarium of Benin of the University of Abomey Calavi under the voucher number YH515/HNB.

Animal material

Non-pregnant and three-month-old female Wistar albino rats weighing between 150-200 g from the Institute of Applied Biomedical Sciences of Cotonou (ISBA) were used in this study. These rats were randomly divided into two groups of three (3) animals each and acclimatized for 2 weeks period at the Laboratory of Physiology and Pharmacology of the Faculty of Sciences and Techniques of the University of Abomey-Calavi before the experiment. During this period, the animals had free access to food and water. These animals (Wistar rats) were maintained in the pet store at room temperature of 20–26°C as well as a regular light cycles of 12 hours light/dark.

Methods

Preparation of the ethanolic extract of the leaves of *Opilia celtidifolia*

Fresh leaves of *Opilia celtidifolia* were collected and cleaned with tap water and then dried at ambient temperature at the Laboratory of Physiology and

Pharmacology of the Faculty of Science and Technology of the University of Abomey-Calavi. After drying, leaves were then reduced in powder using an electric mill and used for the ethanolic extract prepared according to the methodology applied by Klotoé *et al.* (2020). Briefly, fifty (50) grams of the powder of the leaves of *Opilia celtidifolia* were macerated in 500 mL of ethanol. After 72 hours of agitation of the mixture continuously at room temperature, the homogenate was filtered thrice on absorbent cotton and once on Whatman filter paper. The filtrate obtained was then evaporated at a temperature of 40°C in an oven (drying oven) to obtain the crude extract. The extract thus produced was placed in the refrigerator at 4°C and put back into solution for the experiment.

Acute oral toxicity

Two groups of rats (test and control) were constituted according to their body weight. Rats from the control group were received only with distilled water (1mL per 100 g). Those from the test group received by esophageal gavage 5000 mg per kg of bodyweight of the crude ethanolic extract of *Opilia celtidifolia* leaves accordingly to the method described in OECD guideline 423 related to acute toxicity class method (OCDE_423, 2002). The studied plant is commonly used by the population without mention of toxicological effects and this justifies the limit dose choose for this experimentation. Rats were deprived of food and water twelve hours before the beginning of the toxicity test. During the experiment, animals were monitored and observed individually twice a day (morning and evening) over 14 days. A data collection sheet was drawn up for each rat to collect the clinic's signs of toxicity (skin and hair changes, oedema, walking backwards, breathing difficulties, morbidity, and mortality). Rats were once again deprived of food the last night before sampling (day 15). Blood samples have been collected at the retro-orbital sinus of the rats anaesthetized with thiopental (60 mg/kg) to evaluate biochemical and hematological parameters. Moreover, the histological study of the kidneys and liver was carried out at the Histopathology Laboratory of the Institute of Applied Biomedical Sciences (ISBA) of the University of Abomey-Calavi.

Data analysis

Data generated by this study were subjected to statistical analysis using SPSS 26.0 and Microsoft Excel 2016 spreadsheet software. Quantitative variables are presented as means and standard deviations. The test *t* of Student was used to compare the means of the different parameters between the two groups. The significance threshold was set at 5%.

Results and discussion

LD_{50} of the extract of the studied plant

No mortality was recorded for the two groups. Similarly, no signs of apparent toxicity were observed. These data are comparable to the data reported by Konaté *et al.* (2014) on the aqueous acetone extract of the leaves of the plant on mice. Nevertheless, these authors reported that the LD_{50} of this extract in intraperitoneal administration was 636.2 mg/kg body weight with some signs of toxicity observed in mice but which were reversible over time. Our present result suggests that, in oral administration, the lethal dose of the ethanolic extract of the leaves of O*pilia celtidifolia* is higher than 5000 mg/kg.

Table 1. Effect of extract on biochemical parameters of Wistar rats.

Parameters	Control	Test
Urea (g/L)	0.37 ± 0.05	0.66 ± 0.59
Creatinemia (mg/L)	6.26 ± 0.57	6.25 ± 0.43
ASAT (UI/I)	186.81 ± 29.43	172.97 ± 41.76
ALAT (UI/I)	94.68 ± 29.49	103.41 ± 30.5
Total Bilirubine (mg/L)	2.08 ± 0.74	2.45 ± 0.4
Combined Bilirubine (mg/L)	0.68 ± 0.16	0.81 ± 0.13
Alkaline Phosphatase (UI/I)	25.25 ± 6.18	23.5 ± 4.65

Evolution of body weight of treated and control rats

Fig. 1 shows the evolution of the body weight of the rats of the control and test groups during the experimental period. It's come out from this figure that the weight of rats growth in both groups during the experiment.

Table 2. Effect of extract on hematological parameters in Wistar rats.

Parameters	Control	Test
NR (10^6/mm^3)	7.13 ± 0.2	7.01 ± 0.39
HB (g/dL)	13.65 ± 1.06	14.15 ± 0.35
HTE (%)	0.51 ± 0.01	0.48 ± 0.02
CCMH (g/dL)	26.95 ± 2.76	24.8 ± 5.8
VGM (fl)	71.1 ± 0.14	68.95 ± 0.78
TCMH (pg)	14.15 ± 5.02	20.25 ± 0.64
NB (10^3/mm^3)	5.75 ± 1.48	6.6 ± 0.99
PN (%)	15 ± 7	18 ± 1
L (%)	80 ± 6	77 ± 3
M (%)	5 ± 1	4 ± 3
Pl (10^3/mm^3)	605 ± 94.75	756 ± 65.05

NR: Number of Red Blood Cells; HB: Hemoglobin; HTE: Hematocrit; CCMH: Mean Corpuscular Hemoglobin Concentration; VGM: Mean Cell Volume; TCMH: Mean Corpuscular Hemoglobin Content; NB: Number of White Blood Cells; PN: Polynuclear Neutrophils; L: Lymphocytes; M: Monocytes; PI: Eosinophilic Polynuclear Cells.

The comparison of the weight between the two groups did not reveal any significant influence of the treatment on the weight of the Wistar rats ($p>0.05$). Body weight variation is one of the parameters that help to appreciate the toxicological effects of chemical compounds (El Hilaly *et al*., 2004). The data obtained for this parameter in the present study show that rats that received the ethanolic extract of the leaves of *Opilia celtidifolia* reflect the good physiological condition of Wistar rats at the end of the experiment. This observation can be explained by the consumption of food by the rats (Betti *et al*., 2012).

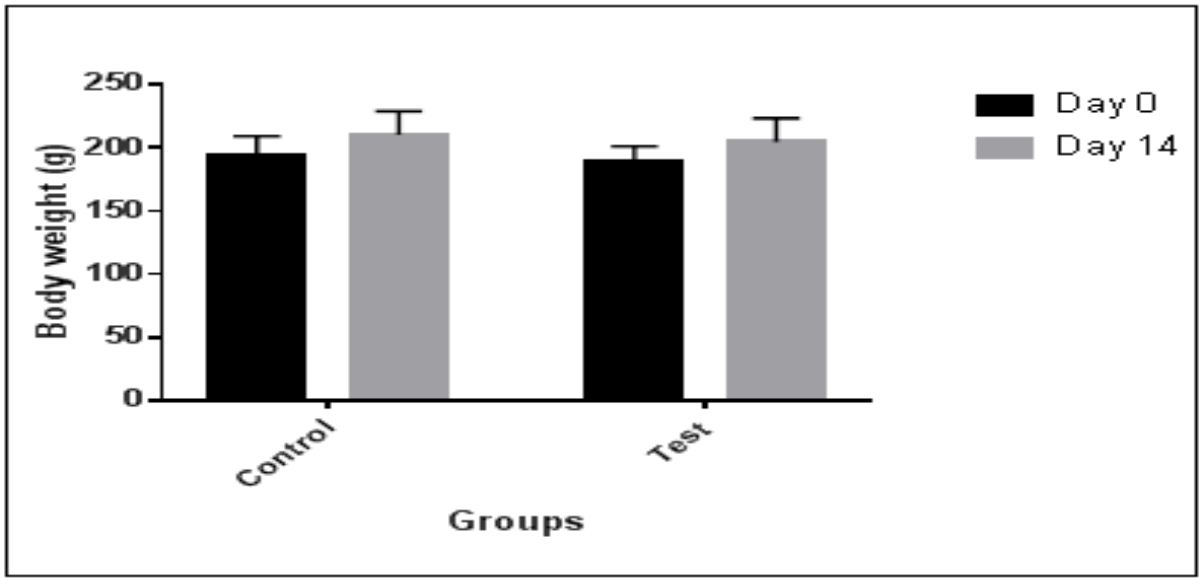

Fig. 1. Body weight of the rats of the control and test groups.

Effect of the ethanolic extract of the leaves of Opilia celtidifolia on biochemical and hematological constants

Table 1 summarizes the influence of the extract evaluated on the biochemical parameters of Wistar rats. Comparison of data in control and test groups shows that the administration of 5000 mg of the extract per kg of body weight did not induce any significant effect on the renal and hepatic biochemical parameters evaluated ($p>0.05$). ALAT and ASAT are

the important markers of liver function. The increase of these enzyme levels in serum, particularly ALAT, suggests damage to the liver (Amacher, 2002; Ramaiah, 2007). In this study, the absence of significant influence noted on these liver parameters reflects that the ethanolic extract of the leaves *Opilia cetidifolia* had no negative impact on the liver function of Wistar rats. In addition, no significant alteration of the renal parameters (urea, creatinine) was recorded in this study.

These results suggest that the tested extract, therefore, did not alter the renal function of the treated rats because it is reported that a significant increase in the rate of these markers translates to an attack of the tissue renal (Yuliandra *et al.,* 2015). Table 2 provides the hematological parameters data for the rats of two groups (test and control). Compared to the control lot, it should be noted that treatment with *Opilia celtidifolia* extract had no significant effect on the white cell line nor red cell line of Wistar rats ($p>0.05$). These observations suggest that the extract tested did not influence the hematopoietic system which is targeted by the toxic molecules (Manda *et al.,* 2017). Any alteration in hematological parameters could be allowed to suspect a potential risk of anemia or inflammation (Mukinda and Syce, 2007).

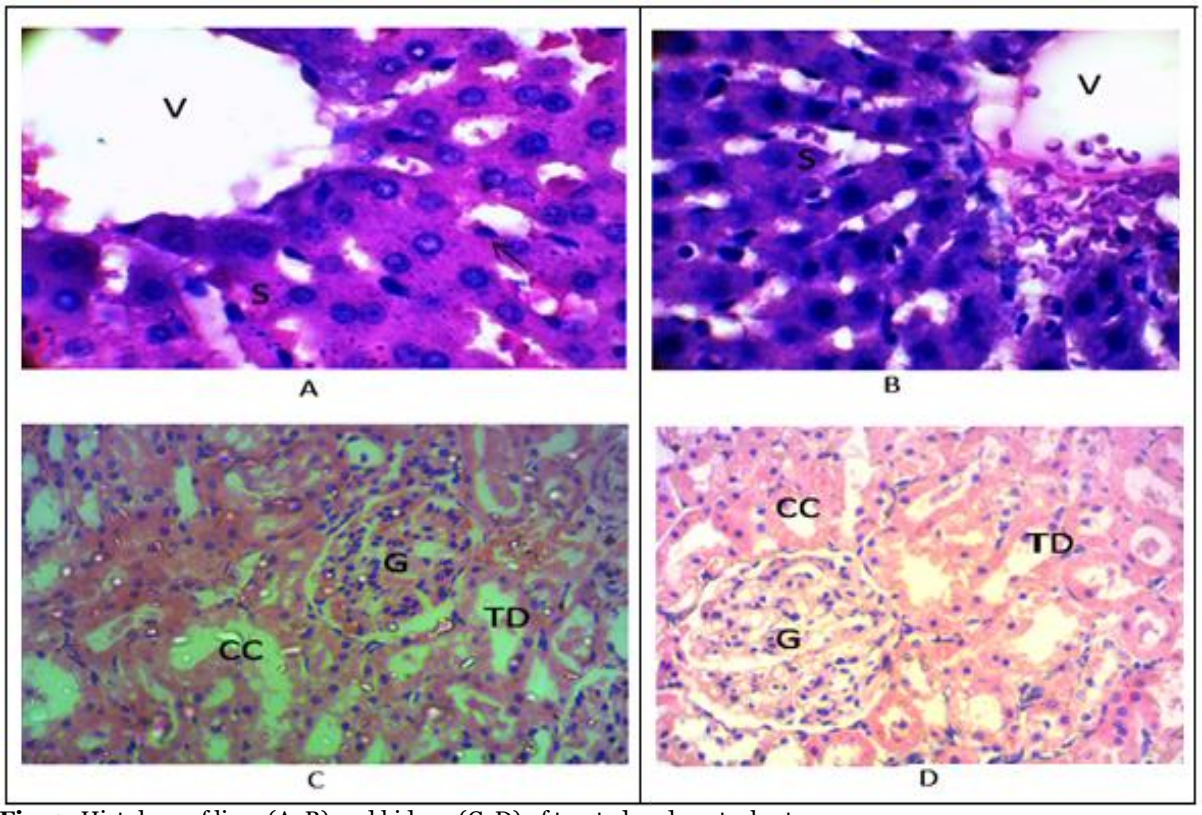

Fig. 2. Histology of liver (A, B) and kidney (C, D) of treated and control rats.

Legend: A: Control group and B: Test group for the Histology of liver. Sinusoids (S); Centrilobular vein (V).

C: Control group and D: Test group for the Histology of kidney. Glomeruli (G), distal tubes (TD) and collecting channels (CC).

Histological study

Fig. 2 shows the histology of liver and kidney of treated and control rats. From this figure, it is recorded hepatocytes (arrows) are separated by the sinusoids (S) which are arranged around the centrolobular vein (V). This organization thus described is typical for both control group and test group rats. For the renal histology, data obtained showed that rats treated with the extract had renal parenchyma of typical architecture as compared to the control group. The glomeruli (G), distal tubes (TD) and collecting tube (CC) are well identifiable.

The tested extract, therefore, did not affect the renal and hepatic structures. These observations confirm the absence of functional impairment of these organs (liver and kidneys) observed in this study.

In the literature, few preclinical studies have explored the degree of safety of *Opilia celtidifolia* leaves. This scarcity of scientific data on the toxicity of the plant despite the long period and frequency of use of its leaves suggests its safety. This study provides scientific evidence that validates this hypothesis by providing recent data on the toxicological profile of *Opilia celtidifolia* leaves, which are the most widely used part of the plant in traditional African medicine.

Conclusion

This study focused on the evaluation of the acute toxicity of the ethanolic extract of the leaves of *Opilia celtidifolia*. The treatment of Wistar rats with the extract of the leaves of the plant did not induce any mortality or any structural alteration of the liver and kidneys. These observations were confirmed by biochemical and hematological parameters. This study proved the safety of the ethanolic extract of the leaves of *Opilia celtidifolia* and thus justified its use in traditional medicine in the treatment of many diseases. Subsequent studies will reveal the toxicological characteristics of its use over a long period in repeated doses.

Ethical considerations

Ethics Committee of the Doctoral School of Life and Earth Sciences of the University of Abomey Calavi of Benin (UAC-Benin) approved this study under the number 10185509.

Conflicts of interest

No conflict of interest.

Author's contributions

All authors contributed to the design, reading and editing of the manuscript.

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