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Hepatoprotective Effect Assessment of the Aqueous Extract of *Crossopteryxfebrifuga* (Rubiaceae) Leaves Benth in the WistarRat

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ABSTRACT

Hepatitis is a global public health problem that causes deaths every year. It represents a heavy burden for health systems because of itsprevalence prospects, itsevolving risks towards complications and their unassessed overall cost. Thus, the objective of this work is to assess the hepatoprotective effect of the aqueous extract of *Crossopteryxfebrifuga* on the biochemical and histological parameters ofintoxicatedrats with carbon tetrachloride. Intraperitoneal intoxication of the extract was assessed by the method of Chacko [11]. It emerges from this work that at doses of 100 mg/kg and 120 mg/kg the aqueous extract of *Crossopteryxfebrifuga* leaves has no toxic effect on body weight of rats, significantly decreases biochemical parameters. It dramatically reduces hepatocyte lesions at a dose of 120 mg/kg. Phytochemical analysis of *C. febrifuga* leaves by the tube method showed the presence of alkaloids, anthraquinones, flavonoids, saponosides, oses, tannins and mucilages.

Keywords:*Crossopteryxfebrifuga*, hepatoprotector, carbon tetrachloride, biochemical parameters, hepatocyte lesions.

I. INTRODUCTION

Hepatic diseases also called hepatopathiesis the severe degradation of hepatic function. Several cases of hepatic diseases are listed today, namely hepatitis, steatosis, cirrhosis, liver cancer, Wilson's disease, hemochromatosis, and ascites [1,2]. These are caused by excessive alcohol abuse, virusesinfection, liver cells dysregulation, excess fat in the liver, iron overload in liver tissue, and severe self-medication with paracetamol, antibiotics, psychotropic drugs, lipid-lowering drugs, non-steroidal anti-

inflammatory drugs [3]. Treatments for hepatic diseases exist, however their prices are very high in developing countries. As a result, about 80% of the population, mostly African, lean towards traditional medicine for primary health care [4]. In addition, work on traditional medicine has shown the importance of medicinal plants in the treatment of certain infections [5]. In this perspective, finding a medicinal plant against hepatic diseases would be beneficial for thousands of lives in developing countries, especially in Congo where cases have not yet been listed [6]. Previous chemical studies of *Crossopteryxfebrifuga* leaves have shown the presence of flavonoids, saponins and tannins in these leaves [7]. These substances are recognized to have hepatoprotective properties [8]. This is why we are interested in *C. febrifuga*leaves Benth and the Rubiaceae family particularly. Traditional therapeutic uses are assumed by traditional healers and some have been scientifically proven [9].

II. MATERIALS AND METHODS

A. Material

- **Plant Material**

The leaves of *Crossopteryxfebrifuga* used were collected in the Kinkala District (Department of Pool) on March of 2019. These leaves were subsequently dried at ambient temperature (26 ± 1 ° C), then sprayed. It is the resulting powder stored in a tightly closed sterile glass jar protected from light and moisture.

- **Animal Material**

Male and female rats albinos strain (weight: 150 - 200g), reared under standard conditions with free access to food and drinking water.

B. Methods

- **Aqueous extract preparation**

75 g of powder from *C. febrifuga*leaves were introduced into a glass flask containing 750 mL of distilled water and then brought to the boil for thirty minutes on a flask heater at a temperature of 100 ° C. The decoction was filtered with cotton wool. The filtrate thus collected was then returned to the flask heater at a reduced temperature of 60 ° C. The resulting dry extract was used to prepare the test solution.

C. Phytochemical analysis of *C. febrifuga* leaves

The phytochemical screening of the extracts allows the identification of different chemical groups of pharmacological interest present in the extract. It was done using the liquid medium characterization methods of alkaloids, anthraquinones, flavonoids, mucilages, saponosides and tannins.

D. Hepatoprotective activity

- **Effect assessment of aqueous extract of *Crossopteryxfebrifuga***

Given the great diversity of study protocols noted in international publications concerning the determination of hepato-protective properties we favored the method of Chacko[10].Groups compound by 6 rats each one wereformed and treated orally once daily for six days as follows: The negativecontrol group (1, 2) received 0.5 ml/100g of distilled water per os. The positive control group (3) is treated with Legalon R (reference molecule) at a dose of 100 mg/kg. The test group (4,5) are treatedwithaqueousextract of *C. febrifuga*at doses of 100 and 120 mg/kg of body weight. On the seventh day after all treatments the rats in groups 2, 3, 4 and 5 were poisoned by intraperitoneal injection of CCL4 (0.5 ml / kg). On the tenth day all the animals of the different groups were sacrificed by decapitation after being anesthetized with ether, the liver and blood were taken for histological and biochemical examinations respectively.

E. Evaluation on biochemical parameters

The serum from rats blood collected previously was used for direct bilirubin analysis (BD), alkaline phosphatase (PAL/ALP) and transaminases (ALAT/GPT; ASAT/GOT) according to manufacturer CYPRESS recommendations.

F. Effects assessment of aqueous extract of *C. febrifuga*leaves in liver histology.

It includes a macroscopic observation of whole livers limited to the external characteristics of the liver and a microscopic examination of livers sections from experimental animals [11]. The livers taken beforehand are fixed in 10% formalin and then included in paraffin wax after dehydration in five successive baths of increasing alcohol (70 °, 80 °, 90 °, 95 °), have been used.The paraffin blocks obtained were stored at ambiant temperature in order to make the histological sections.The histological sections at 4 µ of the paraffin blocks were made with a microtome, then placed in an oven at 37 ° C for 24 hours.One type of coloringwas performed ashematoxylin-eosin (HE) stain.

III. RESULTS

G. Morphometric parameters

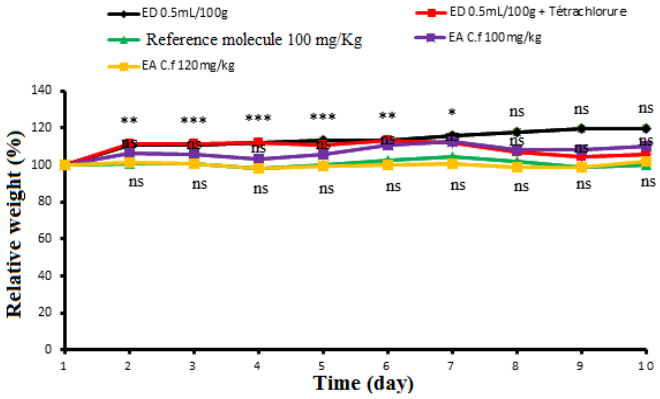

Fig. 1Weight evolution of rats as a function of time: N = 6;* p ≤ 0.5** p ≤ 0.01;*** p ≤ 0.001 significant difference compared to negative control;ED = distilled water;EA = aqueousextract of *C. febrifuga* leaves on biochemical parameters

TABLE I Aqueousextracteffectof *C. febrifuga*leaves on biochemical parameters

Biochemical Parameters	group1	Group 2	Group 3	Group 4	Group 5
ASAT (UI/L)	323,98 ±41,63	512,23 ± 69,15#	256,32 ± 77,83*	288,48 ± 49,92**	252,96 ± 40,89*
ALAT (UI/L)	57,24 ± 17,14	248,67 ± 87,27###	66,00 ± 16,49***	63,91 ± 17,30***	70,42 ± 7,69***
PAL (UI/L)	206,37 ± 37,52	541,02 ± 62,00###	185,75 ± 41,49(N.S)	240,99 ± 16,93***	241,69 ± 72,18**
BT (mg/dL)	0,83 ± 0,60	0,69 ± 0,01(N.S)	0,60 ± 0,65(N.S)	0,67 ± 0,66(N.S)	0,28 ± 0,25(N.S)

Values are means ± ESM, with n = 6;### p < 0.001 significant differences compared to batch 1 treated with distilled water; *** p<0.001 significant difference compared to group 2 treated with distilled water then intoxicated with CCl4. NS: non-significant difference. ASAT and ALAT are transaminases, PAL is alkaline phosphatase, BT = Direct Bilirubin Effect of *C. febrifuga* aqueous extract on liver histological parameters.

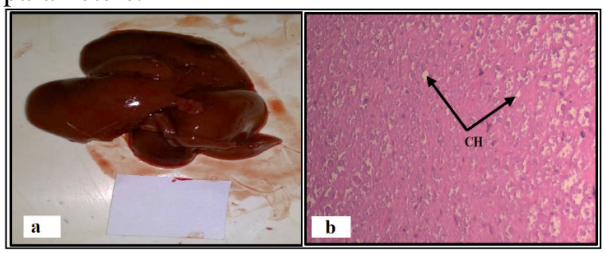

Fig. 2Normal control group image (a: macroscopic view and b: stained with HE. Gx10) CH: hepatocyte clarification

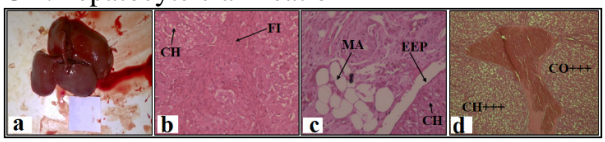

Fig. 3 Control group intoxicating by carbon tetrachloride (a: macroscopic view, b, c and d: colored with HE. Gx20). CH: hepatocyte clarification, FI: fibrosis

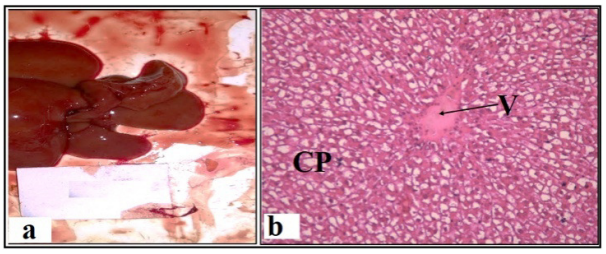

Fig. 4 Control group treated with reference molecule (Légalon R) colored with HE.Gx20 V = centrilobular vein;CP = pericentrolobular clarification

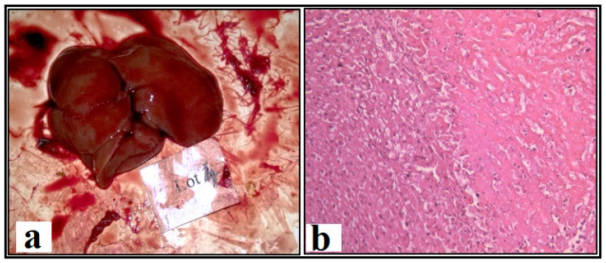

Fig. 5 Group treated with aqueous extract of *C. febrifuga* at a dose of 100 mg/kg then intoxicated with carbon tetrachloride (a: macroscopic view, b: stained with HE Gx40. Extensive coagulation necrosis.

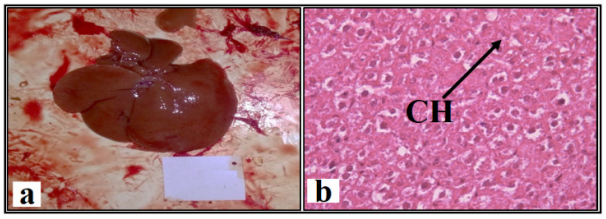

Fig. 6 Group treated with aqueous extract of *C. febrifuga* at a dose of 200 mg/kg then intoxicated with carbon tetrachloride (a: macroscopic view; b: stained with HE Gx40 CH = clarification

F. Phytochemical analysis of aqueous extract of *C. febrifuga*

TABLE 2 Results of chemical screening

Chemical compounds	Observations	Results
Anthraquinone	Red coloring	+++
Alkaloids	Above red or yellowish	+++
Flavonoids	Orange color	+++
Saponosides	Saponosides Moss from 1 to 9 cm	+++
Blackish	Blue tannin	+++
Oses	Red coloring	++
Precipitated	Fluffy mucilage	+

+: Not very abundant;++: Abundant;+++ very abundant.

IV. DISCUSSION

Results of morpho-metricparametersobservedshowed an increase in animalsweight in the first six days. This couldbe due to foodconsumption and continuous water intake. However, on the seventhdayafter animal intoxicatedat CCl4 wefound a non-significantdecrease in animalsweighttreatedwithdistilled water, silymarin and the aqueous extractof *C. febrifuga*at doses of 100 and 120 mg/kg. This decreasewillbe due to CCl4toxiceffect. However, the aqueousextract of *C. febrifuga*did not lead to a decrease in animalsweight. Theseresultssuggestthat*Crossopteryxfebrifuga*would not have toxiceffects in rats. The different*C. febrifuga*familiesare believed to beresponsible for the effectobserved.

Administration to rats of different countries showed a verysignificantincrease in the rate of GOT ($p<0.001$), GPT ($p<0.001$), PAL ($p<0.001$) of group 2 treatedwithdistilled water thenintoxicatedwith CCl4 and the non-significantdecrease in direct bilirubincompared to group 1 treatedonlywithdistilled water (negative control). The increase in GOT, GPT and PAL indicateshepatic damage in rats. Because, the internalized administration of CCl4 isresponsibleto an increase in transaminases and alkaline phosphatase in the blood . On the other hand, the decrease in direct bilirubinsuggeststhat CCl4 did not damage the gallbladder.

For animalstreatedwithaqueous extractof *C. febrifuga* and the referencemolecule, therewas a significantdecrease in the rate of GOT ($p<0.001$), GPT ($p<0.001$), PAL ($p<0.001$). The decrease in biochemicalparameters in rats suggests a process of restoration of hepaticfunction by silymarine and extractatdifferent doses. This suggeststhat*C. febrifuga*extractwould have the ability to reducedamagingeffects or

preventhepaticdysfunctionagainsthepatotoxindist urbances[14]. *C. febrifuga*wouldtherefore has an hepathoprotectiveeffect and the decrease in bilirubinsuggeststhat CCl4 does not act on the gallbladder.

Macroscopicexamination of histologicalcuts of negative control group1 animals shows a normal livercharacteristics: firmliver, crumbly polylobe and withoutorange peelappearance.

Liversmacroscopic observation of animalstreatedwithdistilled water and thenintoxicatedrevealed an accentuatedappearance of orange peel. This resultconfirms the action of CCl4where thehepatotoxicwhich has a mandatory and predictable action on the liver[12]. Animalstreatedwithaqueous extractat all doses thereis a smallappearance of orange peel as well as in thosetreatedwithmoleculereference. This resultsuggeststhat*C. febrifuga*would have hepatoprotectiveeffect. Since the lowappearance of orange peelindicates the regeneration of the process of repairing the tissue lesions[13].

Regardingmicroscopic observation of histologicalcutswefound in animalstreatedwithdistilled water (negative control) a clarification not sufficientlypronounced as well as minimal congestion of connective tissues. This couldbejustified by environmental conditions and changes in food composition.

Histologicalcutting of animalsliversthatreceiveddistilled water and thenintoxicatedrevealedischemia, pronouncedcell clarification, fat metaplasia, verysevere congestion, fibrosis and widening of space. Theselesions are related to massive alterationcaused by toxic substances athepaticlevel. This canbejustified by a verysignificantincrease ($p<0.001$) in biochemicalparameters (ALAT, ASAT and PAL) in relation to negative control.

Animalslivertreatedwithaqueous extractof *C. febrifuga*at the 100 mg/kg dose has extensive clottingnecrosis and single-cellednecrosis. However, wefound a cellsclarification in animalstreatedwithaqueousextractat dose of 120 mg/kg as well as in thosetreatedwithreferencemolecule. This assumes that*C. febrifuga*extract has hepatoprotectiveeffectdepending to dose directedagainst CCl4 intoxication. The presence of flavonoids (alkaloidsaponosides and tannins in C. febrifugawouldberesponsibletoeffectobserved[3].

Chemical profile of *C. febrifuga*leaves in previousworkhadrevealed the presence of thesesecondarymetaboliteswhich are trappers of free radicalsresponsibletohepatic lisions. This reduction in hepaticlesionsmaybejustified by the relatively high levels of polyphenolic compounds in the extractreported in thisstudy and by someauthors[15]. The hepaticlesionsdecreasewouldjustify the traditional use of aqueous extract of *C. febrifuga*leaves. Phytochemicalanalysis of aqueous extract of *C. febrifuga*leavesrevealed the presence of three main chemical groups asflavonoids, saponosides and tannins known to have hepatoprotectiveproperties[3,8,13]. In addition to thesedifferentfamilieswe have highlighted the presence of four otherfamilies as anthraquinones, alkaloids, dares and mucilages. *C. febrifuga*couldtherefore have ahepatoprotectiveeffect.

V. CONCLUSION

Aqueous extract of *C. febrifuga*leaves shows an opposition to biochemical parameters disturbance (transaminases, PAL and BD), preventing the hepatic lesion onset in intoxicated rats with Ccl4.These observations can be attributed to hepatoprotective activity against Ccl4 intoxication in rats.The proof of this activity is to reduce lesions observed in animals treated with aqueous extract at different doses during animal livers histology.However, the effect could be due to the presence of several secondary metabolites present in *C. febrifuga*leaves namely alkaloids, flavonoids and saponosides.Further studies should also be carried out in order to determine

the mechanism of thisplant action to achieve phytomedicinemanufacture.

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