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Bamba Abou
Laboratoire de Pharmacodynamie-Biochimique, UFR Biosciences, Université FELIX Houphouet Boigny de Cocody-Abidjan (Côte d'Ivoire), 22 BP 582 Abidjan

Yapi Houphouet Félix
Laboratoire de Pharmacodynamie-Biochimique, UFR Biosciences, Université FELIX Houphouet Boigny de Cocody-Abidjan (Côte d'Ivoire), 22 BP 1679 Abidjan

Djyh Bernard Nazaire
Laboratoire de Pharmacodynamie-Biochimique, UFR Biosciences, Université FELIX Houphouet Boigny de Cocody-Abidjan (Côte d'Ivoire), 22 BP 582 Abidjan

Correspondence:
Yapi Houphouet Félix
Laboratoire de Pharmacodynamie-Biochimique, UFR Biosciences, Université FELIX Houphouet Boigny de Cocody-Abidjan (Côte d'Ivoire), 22 BP 1679 Abidjan

Determination of hematological parameters and Biochemical markers of kidneys and liver in the acute toxicity of *Gomphrena celosioides* ethanol extract

Bamba Abou, Yapi Houphouet Félix, Djyh Bernard Nazaire

Abstract
The limited data on the toxicity of medicinal plants leads us to continue researches to increase and / or improve the results available, in order to better appreciate the therapeutic doses to use. This study aimed at determining the biochemical and hematological profiles during the acute oral toxicity of ethanol extract of *Gomphrena celosioides* in rats.
The acute oral toxicity was evaluated in rats according to the guideline n^0 423 of the OECD. Under this method, the starting dose is selected from these four, 5, 50, 300 and 2000 mg / kg. The level chosen is the one for which we can expect to see mortality among treated animals. The absence of treatment-related mortality at the initial dose determines the next step. The animals were daily observed for 14 days after treatment. On the fifteenth day, the animals were killed and dissected. Their blood and organs (heart, kidneys and liver) were collected for biochemical and hematological analyzes.
The results showed that the LD50 by oral route in rats was greater than 5000 mg / kg body weight. The relative weights of organs (kidneys, liver and heart) and hematological parameters were not significantly different (P> 0.05). Biochemical analyzes showed a significant increase in transaminases ALT and AST (P<0.05) at a dose of 5000 mg / kg.
This study suggests that the indiscriminate use of the ethanol extract of *Gomphrena celosioides* should be avoided because with the dose of 5000mg / kg, it would induce hepatic dysfunction.

Keywords: Acute toxicity, *Gomphrena celosioides*, biochemical markers , liver and kidneys

1. Introduction
A Medicinal plant is a plant used for its specific properties beneficial to human or animal health. These medicinal plants are an effective source for both modern and traditional medicines, and about 80% of the rural populations depend on them for their primary health care's [1]. They are used in different ways (decoction, maceration, infusion ...). However, the toxicity of plants from botanical data was underestimated due to the perception that herbal medicines are absolutely safe. However, severe hepatic dysfunction has been reported after ingestion of a large variety of different herbal preparations [2]. A determination of the efficiency and safety of herbal remedies is necessary because many people use them for self-medication and little is known about the pharmacology and toxicology of the most common remedies [3] .This determination is made as to highlight potential dangers for human health associated with the use of these medicines.
Gomphrena celosioides is a plant of the family of Amaranthaceae. It is an annual or perennial, pubescent, spread Swivel, woody roots, being about 20 cm high, reproducing from seeds. The aqueous extract of *Gomphrena celosioides* revealed the presence of sterols and triterpenes, flavonoids, saponins, coumarins and tannins [4]. In Nigeria, this plant is used for the treatment of various skin conditions [5]. It also has analgesic, tonic, carminative and diuretic [6]. In Benin, traditional healers make use of this plant (aqueous extract) in the treatment of jaundice, malaria and dysmenorrhea [7].
Despite these many virtues, very little work has been done on the ethanol extract of *Gomphrena celosioides*. Furthermore no data on the effect of acute oral toxicity on hematology and biochemical markers of liver and kidneys are available. This study was conducted to determine the biochemical and hematological changes during acute oral toxicity of *Gomphrena celosioides* ethanol extract leaves in female albino rats.

2. Materials and Methods

2.1 Materials

2.1.1 Plant material:

Gomphrena celosioides was harvested in Bingerville in the District of Adidjan. A sample of this plant has been authenticated by the UFR's (Training and Research Unit) Laboratory of Botany and Plant Biology- Biosciences of Felix Houphouet Boigny University of Cocody-Abidjan.

2.1.2 Animal material:

The animal species chosen for this study was the albino rat (Rattus norvegicus) of the Wistar breed. The rats were bred in the animal facility of Higher Normal School (ENS) at room temperature. In these premises, the photoperiod was 12 hours and the animals had free access to water and food. Nine young female rats, aged 10-11 weeks, average weight 109.82 g were used for the experiments. The selected rats were nulliparous, non-pregnant and had not been the subject of previous studies.

2.2 Methods

2.2.1 Preparation of the ethanol extract:

The plant was dried in the open air, protected from light, at room temperature (25^0 c) for two weeks, and then ground using an electric grinder. The extract was prepared by macerating 100 g of powder of *Gomphrena celosioides* in 2 l of water / ethanol (30/70 v / v) using a magnetic stirrer. The macerated was then sieved, filtered through cotton wool and then on filter paper (Whatman No. 1). The filtrate obtained was finally dried in an oven at 50 ° C. This yielded 12.25 g of dry extract, which corresponds to a yield of 12.25%.

2.2.2 Study of acute toxicity by gavage:

It was carried out in accordance with OECD Guideline n^0 423 for testing of chemical substances adopted on 17 December 2001 [8-9]. The principle of this test is that with a sequential process, using by-step a minimum number of animals (3), information on the acute toxicity of the substance are obtained and are sufficient for classification. The study was performed using an intragastric sonde in female rats. The animals were divided into 3 batches of 3 rats each. Batch1 (control) was treated with saline solution (NaCl 9 ‰). Batch2 received the ethanol extract of *Gomphrena celosioides* orally at a dose of 2000 mg / kg. This initial dose administered as a single dose was chosen from the following four doses: 5, 50, 300 and 2000mg / kg so that one can expect to see mortality among some of the treated animals. After a 48-hour observation, without toxicity reaction, group 3 (experimental) received the extract at a dose of 5000 mg / kg. The toxicological effects were observed in terms of changes in the skin, hair, the somatomotor activity and behavior. Particular attention has been placed on the observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and death. These observations allow us to classify our sample into a category of globally harmonized classification system (GHS) of chemical substances (OECD, 2001).

2.2.3 Samples:

After 14 days of observation, all test animals were euthanized at the end of treatment. And organ collections were made (liver and kidney heart). The blood of each animal was taken (puncture of the orbital sinus) both in a tube containing anticoagulant (EDTA) and in a tube without anticoagulant, for respectively metering the haematological and biochemical parameters. The organs were washed in normal saline, weighed and preserved in Bouin. The relative organ weight was determined by the following formula:

Relative Body weight (%) = (absolute body weight / animal body weight) x 100 [10].

2.2.4 Blood tests:

Blood samples collected in tubes containing anticoagulant (EDTA) were immediately used to determine the levels of white blood cells, red blood cells, platelets and hematocrit using an automated type of Sysmex XT 1800 I.

2.2.5 Biochemical Study

Blood samples collected in anticoagulant-free tubes were centrifuged at 3000 rev / min for 15 minutes. The collected sera were used to assay the biochemical parameters are: alanine aminotransferase (ALT), aspartate aminotransferase (AST), Total and conjugated bilirubin, total cholesterol, triglycerides, creatinine and urea through an automated type of Cobas C311 HITACHI of ROCHE.

2.2.6 Statistical analysis:

Statistical analyzes of the results were performed using STATISTICA 7.1 software. Values are presented as average ± standard deviation. The degrees of significance between treated batches and control rats were measured by the Newman Keuls test. If $p < 0.05$, the difference between the values was considered significant.

3. Results and Commentaries

3.1 Reesults

Table 1: Effect of ethanol extract of *Gomphrena celosioides* on the general appearance of the animals

Duration	1/2 hours	4 hours	24 hours	48 hours	96 hours	144 hours	336 hours
Control	NRT	NRT	NRT	NRT	NRT	NRT	NRT
Batch G1	NRT	NRT	diarrhea	diarrhea	NRT	NRT	NRT
Batch G2	NRT	NRT	LA	LA	NRT	NRT	NRT

NRT: nothing to report, LA: lack of appetite, batch G1: animals treated with the ethanol extract of *Gomphrena celosioides* at a dose of 2000mg / kg and Batch G2: animals treated with the ethanol extract of *Gomphrena celosioides* 5000 mg / kg Controls: animals treated with NaCl.

Observations made during the test period indicate that the animals showed no change in the overall appearance and the somatomotricity. Also no manifestations of tremors, convulsions, salivation, diarrhea, coma or abnormal behaviors such as self-mutilation or walking backwards were observed. However, animals treated with a dose of 2000mg / kg (Batch G1) of the ethanolic extract of *Gomphrena*

celosioides had very smooth waste after 24 hours and 48 hours while those treated with a dose of 5000mg / kg of the same extract showed lack of appetite in the same period.

Table 2: Actual weight through the heart, liver and kidneys of rats 14 days after a single administration of the ethanol extract of *Gomphrena celosioides*.

Rats	Control	Batch G1	Batch G2
Heart	0.68 ± 0.13	0.57 ± 0.049	0.46± 0.006
Liver	5.81 ± 0.9	5.82 ± 0.9	4.25 ± 0.11 *
Kidney	1.09 ± 0.14	1.04 ± 0.1	0.99± 0.1

*: P< 0.05, Batch G1: animals treated with the ethanol extract of *Gomphrena celosioides* at a dose of 2000mg / kg and Batch G2: animals treated with the ethanol extract of *Gomphrena celosioides* at 5000 mg / kg and Control : animals treated with NaCl

Analysis of organ weight indicates that the animals in the batch treated at a dose of 2000mg / kg have their organs (kidneys, livers, and hearts) of weight statistically identical to those of the witness batch (P> 0.05). However, animals treated at the limit dose (5000mg / kg) have their liver weights statistically lower than the witness batch (P<0.05).

Table 3: Actual average weight of the heart, liver and kidneys of rats 14 days after a single administration of the ethanol extract of *Gomphrena celosioides*.

Rats	Control	Batch G1	Batch G2
Heart	0.53% ±0.1%	0.46% ± 0.05	0.42 ±0.02
Liver	4.48% ±0.27	4.67% ± 0.82	3.81%±0.22
Kidney	0.84%± 0.09	0.84%0.1	0.76% 0.03

BatchG1: animals treated with the ethanol extract of *Gomphrena celosioides* at a dose of 2000mg / kg batch G2: animals treated with the ethanol extract of *Gomphrena celosioides* 5000 mg / kg ,Control: animals treated with NaCl

Table 3 presents the effect of *Gomphrena celosioides* on the relative weight of the heart, liver and kidneys on the total weight of the animal. Analysis of the average of the relative weight of the organs studied shows no statistical difference (P> 0.05) between the control group and the treated groups (2000mg / kg and 5000 mg / kg).

Biochemical and hematological parameters

In this study, transaminases (AST, ALT), blood glucose, cholesterol, total and conjugate bilirubin, and the creatinine, urea and total protein were measured for biochemical parameters. For hematological parameters, we evaluated the number of red blood cells, white blood cells, blood platelets, lymphocyte, neutrophil, the rate of hemoglobin, hematocrit and average corpuscular volume.

Table 4: Effect of acute toxicity of ethanol extract of Gomphrena celosioides on biochemical parameters in rats.

Parameters Studied		Control	Batch G1	Batch G2
Transa	Alat (U/L)	47.13± 6.5	55,10± 4.38	71.83± 5.26 *
	Asat (U/L)	120.6± 9.7	162,4± 0.35	178.5± 18.97 *
Creatinine (Mg/L)		3.33± 0.57	2± 0.01	3 ± 0.01
Urea (G/L)		0.24± 0.06	0.29± 0.014	0.33± 0.026
Cholesterol (G/L)		0.57± 0.025	0.91± 0.20	0.66± 0.10
Glucose(G/L)		1.27± 0.083	1.37± 0.05	1.15± 0.19
Conjugated Bilirubin (Mg/L)		0.12 ± 0.20	0.18± 0.26	0.14± 0.11
Total Bilirubin (Mg/L)		0.76± 0.17	0.9± 0,03	0.86± 0.13
Total Protein (G/L)		60.13± 1.96	67.9± 1.14	61± 3.60

*: P< 0.05, TRANSA: transaminases, ALAT: alanine aminotransferase, ASAT: aspartate aminotransferase.

Upon observation of Table 4, it is discovered that the averages of the biochemical parameters of animals treated with the ethanol extract of *Gomphrena celosioides* at a dose of 2000mg / kg (batch G1) are statistically identical to those of controls (P> 0.05). On the contrary, for animals treated at a dose of 5000 mg / kg there was a significant increase (P<0.05) of transaminases, compared to control rats.

Table 5: Effect of acute toxicity of ethanol extract of *Gomphrena celosioides* on hematological parameters in rats.

Parameters Studied	Control	Batch G1	Batch G2
Red Blood Cells (10^3/Ml)	6.37±1.16	6.2±3.21	8.07±0.12
Hemoglobin (G/Dl)	11.70± 1.56	12.13±2.39	13.5±0.7
Hematocrit (%)	34.57± 5.36	36.93± 10.46	42.09±1,28
Mcv (Fl)	54.40± 3.46	63.87± 12.57	52.43±0,89
Mch (Pg)	18.50± 2.16	22.63±6.09	16.56±0,58
Mchc (G/Dl)	33.93± 1.85	35.00±2.96	32.7±1,07
Platelets (10^3/Ml)	410.0± 355.5	259.7± 294.7	548.33±109.87
White Blood Cells (10^6µl)	6.86± 2.55	6.7± 1.98	6.12±0.38
Neutrophil (10^3µl)	1.69± 0.66	1.40± 0.23	1.29±0.33
Lymphocyte (10^3µl)	4.68± 1.67	4.81± 1.82	4.06±0.39
Monocyte (10^3µl)	0.3± 0.16	0.36± 0.09	0.33±0.04
Eo (10^3µl)	0.18± 0.12	0.19± 0.16	0.15±0.08
Baso (10^3µl)	0.0± 0.0	0.006± 0.005	0±0

MCV: mean corpuscular volume, MCH: average content of hemoglobin, MCHC: average hemoglobin concentration, EO: eosinophil, BASO: basophil.

The analysis of Table 5 shows that the hematology parameters are statistically identical (P> 0.05) to animals treated with doses of 2000mg / kg and 5000 mg / kg of the ethanol extract of *Gomphrena celosioides*.

3. Discussion

There is a growing concern about the toxicity of herbal remedies because they contain significant amounts of active pharmaceutical ingredients whose mechanisms of action and side effects are mostly unknown[11]. Serious liver injuries, including acute and chronic abnormalities have been described after ingestion of a wide range of herbal products [2]. Therefore, the investigation of biochemical alterations and hematological changes associated with acute oral toxicity of ethanol extract *Gomphrena celosioides* was carried out..

Our study on acute toxicity, showed no lethality following oral administration of the ethanol extract of *Gomphrena celosioides* at a dose of 2000mg / kg and the limit dose of 5000 mg / kg body weight. Which means that; by this means, the LD50 is greater than these two doses [10] The LD50 of the ethanolic extract of *Gomphrena celosioides* would be greater than 5000 mg per kg of bodyweight .According to Diezi (1989) [12], substances having a LD50 comprised between 50 and 500 mg / kg of BW are toxic, and those with a LD50 greater than 5000 mg / kg are practically non-toxic. As we referred to this classification, the ethanol extract of *Gomphrena celosioides* is nontoxic orally. This observation allows us to classify our extract in Category 5 of globally harmonized classification system (GHS) of chemicals. A Classification that characterizes slightly toxic substances (OECD, 2001).

The vast majority of exogenous composites administered to

the body are gastrointestinal tract. These composites will therefore be subsequently distributed to the liver to be metabolized before being eliminated by the kidneys [13]: The heart which is also a vital organ undergoes the toxic effect of substances. That is why, although having no rats died following treatment, we focused on these three organs. The analysis of the relative organ weights studied showed that they were all statistically identical to those of Control rats. But comparing actual organ weights, indicates a significant decrease in liver weight at a dose of 5000mg / kg. The liver weight changes may suggest treatment-related changes, including hepatocellular hypertrophy [14]. It appears from this analysis that the liver was certainly affected by the ethanol extract of *Gomphrena celosioides* at a dose of 5000 mg / kg.

Hepatorenal toxicity was studied by measurement of some biochemical parameters of the kidney and liver. With the exception of transaminases, we did not observe a significant difference (P> 0.05) for the values of other biochemical parameters (conjugated bilirubin and total, total protein, blood glucose, cholesterol, creatinine, and urea). ALT is a cytosolic enzyme secreted in the liver cells where it is released into the blood in liver cell necrosis [15-16]. This is a specific enzyme in the liver, making it an important indicator very sensitive to hepatotoxicity [17-18]. ALT is also an indicator of destruction of hepatocytes even if in addition to the liver, it is found in the heart, skeletal muscles, lungs and kidneys [16]: The rate of ALT and AST increases rapidly when the liver is damaged for various reasons including liver cell necrosis, hepatitis, cirrhosis and liver toxicity of certain drugs [17-16]. In our study, the concentration of these two enzymes (ALT and AST) increased significantly (P<0.05) in animals treated with a dose of 5000mg / kg compared to control rats. The tests of liver function led by blood dose gave information on liver status, describing its functionality (albumin), cellular integrity (transaminases) and its relationship to the biliary tract (alkaline phosphatase) [19] . We now understand that it is the integrity of liver cells that is called into question.

A high dose (5000mg / kg) *Gomphrena celosioides* would therefore lead to cell damage in the liver. In sum, we note that the dose of 2000mg / kg test substance would be properly metabolized by the liver and also would do a perfect elimination by the kidneys. It is noteworthy that the ethanol extract of *Gomphrena celosioides* at a dose of 2000mg / kg would have no deleterious effect on the liver and kidney. On the contrary, at a dose of 5000 mg / kg, there would be proper elimination by the kidneys but a metabolism in the liver, which is unsatisfactory. Therefore, the ethanol extract of *Gomphrena celosioides* would not have a toxic effect on the organs studied at doses lower than 5000 mg / kg. Statistical analysis of hematological parameters studied revealed no significant difference (P> 0.05) between those of the control batch and those batches treated with the extract at doses of 2000 mg / kg and 5000 mg / kg body weight. We deduce that the ethanol extract of *Gomphrena celosioides* would have no effect on hematological parameters.

4. Conclusion

This study was to identify potential dangers that the use of *Gomphrena celosioides* leaves would represent for human health; considering its traditional use for the treatment of certain diseases. The acute oral toxicity test in rats yielded an LD50> 5000mg / kg, characteristic of substances of very low toxicity. We did not notice any signs of intoxication, both during the trial period, during the analysis of biochemical and hematological parameters studied. However, at a high dose (5000mg / kg), this plant induced liver dysfunction. It should be noted that at this dose (5000 mg / kg) it would also be the basis for a hepatocellular hypotrophy. These results assume that the use of ethanol extract of *Gomphrena celosioides* doses lower than 5000 mg / kg presents no risk of intoxication.

After this study, it would be possible to identify and elucidate the mechanisms of action of various chemical constituents responsible for its liver toxicity effects.

Or else, carrying out Sub acute toxicity to evaluate the harmful effects of the extract after long-term treatment.

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