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Evaluation of the Toxicity of Leaves of Five Cassava Accessions Collected at SAMBA in Ombella M'Poko in the Central African Republic

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Abstract

Cassava (*Manihot esculenta*) is a perennial shrub of the Euphorbiaceae family. Thus, the objectives assigned to this study are to evaluate the toxicity of the leaves of 5 cassava accessions collected in Samba using mice of the Wistar albino provided by the Pasteur Institute of Bangui. The aim was to evaluate the biochemical parameters and the effects of cyanide on the vital organs of mice in the experiment. Leaf powders from five cassava accessions with concentrations, respectively: 100.66 mg/kg (ICRA14), 103.33 mg/kg (ICRA51), 134.66 mg/kg (MAMBERE), 135.1 mg/kg (ICRA21), and 144 mg/kg (DOGBO) considered higher than the FAO standard (10 mg/kg), were incorporated into the standard diet with a proportion of 25% of the leaf powders from the cassava accessions and 75% of the standard diet. These different food formulations were given daily for 14 days to white mice divided into 6 groups of 6 and a control group (exclusively the standard diet). At the end of the experiment, the mice were sacrificed, and blood and vital organs were taken for various tests, including biochemical tests (ASAT, ALAT, urea, creatinine) as well as histopathological examination of vital organs (liver, kidneys, heart). The re-

sults show a very significant elevation of liver and kidney markers, accompanied by weight loss and visible changes in the organs examined, highlighting the presence of atrophy, dystrophy, ischemia, and necrosis in the livers, kidneys, and hearts of the mice tested, except for the control group. These results also show that the accessions studied, DOGBO, ICRA21, MAMBERE, ICRA51, and ICRA14, have considerable toxic potential. This information is crucial and raises awareness as a prelude to the solutions to be considered. It is therefore crucial to carry out a thorough assessment before any consumption of leaves and derived products, particularly by-products, in the Central African Republic.

Keywords

Cassava, Acute Toxicity, Subacute, Cyanogenesis, Biochemistry, Histology, Central African Republic

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a plant native to Brazil (South America), cultivated throughout the tropics for its starchy roots [1]. It feeds more than 700 million people and is cultivated in more than 105 countries worldwide [2], with a global production of 292 million tonnes in 2017 according to the FAO. Approximately 60% of cassava produced is intended for human consumption, 20% for animal feed, and 10% is processed into secondary products [3]. The leaves are eaten as vegetables in many parts of Africa. In the Central African Republic, cassava production is 725,000 tonnes of cassava, or 2.9 million tonnes of fresh tuber, followed by yams at 450,000 tonnes and maize at 167,000 tonnes. Despite its advantages, cassava is one of the toxic food plants in the world. This toxicity is linked to the presence of two cyanogenic glucosides: linamarin (93%) and lotaustraline (7%) [4]. These glucosides in themselves are not toxic, but following enzymatic hydrolysis, they release hydrocyanic acid [5]. Many studies seem to establish a link between diet and the appearance of certain pathologies. One of the main explanatory factors would be repeated exposure to low doses of toxic contaminants via food. KONZO disease in Congo is due to the consumption of cassava containing a high level of HCN [6]. KONZO is an irreversible neurological disorder affecting the upper body, prevalent in rural areas of Sub-Saharan Africa, where the main crop is bitter varieties of cassava. Due to the dangers of chronic toxicity of this plant, there is talk of more intensive efforts to achieve low and acceptable levels of cyanide. The first possibility for reducing cyanide levels in cassava lies in the selection of cultivars that are free or low in HCN [7]. Cyanide-free varieties are difficult to find, but studies will always continue. Cassava varieties that produce less than 50 mg/kg of HCN from fresh peeled roots are considered safe [8] [9]. The CAR is one of the countries where cassava is the staple food, and its cultivation is widespread throughout the country. The leaves are consumed

as a vegetable and the roots as an energy source. In the Central African Republic, numerous studies have been carried out on its improvement [10] [11]. An epidemiological survey confirmed the presence of KONZO disease (epidermal paralysis induced by chronic poisoning by food-borne cyanide) in health district No. 2 in 2009 [12]. Also, a study reported that during the rainy season, the demand for cassava chips exceeds the supply in most countries. This deficit sometimes leads some processors to voluntarily shorten the processing time of cassava. As a result, the time for retting and drying operations is often shortened. This leads to risks of toxicity due to the persistence of cyanogenic derivatives in the chips. In order to guarantee food safety, it is a question of launching research on the toxicological aspect of this plant. This is why, through this research project, we have already evaluated the HCN content in the leaves of 58 cassava accessions in circulation in the Central African Republic, whose determined HCN concentrations vary from 13 mg to 144 mg, exceeding the FAO standard of 10 mg [13]. This is a worrying situation, because recent work has shown that the frequency of consumption of cassava leaves is 1 to 4 times per week in households [14]. This would expose consumers to a health risk due to the cyanide effect. This requires verification of the effects of the toxicity of the leaves of these accessions on mice, which could help in the search for solutions in humans. This is why this study aims to evaluate the biochemical parameters and effects of cyanide on the vital organs of mice in the experiment.

Research questions

Are fresh cassava leaves toxic? What impact can they have on humans?

General objective

Assess the toxicity of leaves from five cassava accessions in the collection in SAMBA in Ombella M'Poko in the Central African Republic.

Specific objectives

- Evaluate biochemical parameters, including urea, creatinine, and transaminases;
- Search for effects of cyanide on vital organs of mice.

2. Materials and Methods

2.1. Plant Material

Fresh leaves from five cassava accessions grown in experimental plots in Samba (Ombella-M'Poko) were collected. After sorting, washing, and drying in the shade, these leaves were processed into a fine powder and stored for analysis.

2.2. Animal Material

Wistar albino mice weighing between 35 and 55 g were provided by the Pasteur Institute in Bangui. After an acclimatization period, they were divided into six groups, including a control group that received the standard diet and five other groups that received the mixed diet, *i.e.*, cassava leaf powder plus the standard diet with a proportion of 25%/75% [14] (**Table 1**).

The average daily consumption of mice is 63.73 g.

Table 1. Summary table of the experimental phase.

Batch number Witness and the five accessions	Known HCN concentrations in the leaves of five cassava accessions	Mouse group number Having received standard food and mixed food
Batch No. 1 Witness = six mice per cage	Exclusively standard food	Group No. 1 Control receiving 100% of the standard food
Lot No. 2 ICRA14 = six mice per cage	100.66 mg/kg	Group No. 2 Control receiving mixed feed
Lot No. 3 ICRA51 = six mice per cage	103.33 mg/Kg	Group No. 3 Control receiving mixed feed
Lot No. 4 MAMBERE = six mice per cage	134.66 mg/Kg	Group No. 4 Control receiving mixed feed
Lot No. 5 ICRA21 = six mice per cage	135.1 mg/kg	Group No. 5 Control receiving mixed feed
Lot No. 6 DOGBO = six mice per cage	144 mg/kg	Group No. 6 Control receiving mixed feed

2.3. Experimental Protocol

The leaf powders were mixed with the standard diet (at a ratio of 25/75%). Control mice (group 1) received 63.73 g of the standard diet, while mice in groups 2 to 6 each received the mixed diet consisting of **47.87 g of corn flour and 15.93 g of cassava leaf powder. During the experiment**, which lasted 14 days, clinical observations and weighings were made. After the experiment, blood samples were taken to perform biochemical analyses (urea, creatinine, ASAT, ALAT), and mice were sacrificed. The organs (liver, kidneys, heart) were removed for histological study (using Hematoxylin-Eosin staining).

Histological analysis: Organ tissues (such as liver, kidney, and heart) were preserved in formalin, then embedded in paraffin, sliced using a microtome, stained using the H and E method, and examined under a light microscope.

Statistical analysis: The results were analyzed using the GraphPad Prism program. Comparative analyses were performed using the Student test, with the significance level set at $p < 0.05$.

3. Results of the Work Carried out

3.1. Results of Biochemical Parameters

Monitoring of daily consumption of cassava leaf powders mixed with standard food.

The results show that the daily consumption rate increases gradually and reaches a plateau on the 2nd day. The consumption is 20 g on the 1st day and 62 g on the 12th day until the 14th day (Figure 1).

The results show the non-adaptation of mice to the change in diet, causing sig-

nificant weight loss from the 3rd day to the 7th. However, the adaptation phase is observed only from the 9th to the 13th day, which justifies the increase in weight of the mice subjected to the experiment. These disturbances are due to the powders of the leaves of different cassava accessions having various concentrations of HCN. However, the control group did not experience any change in weight variation from the beginning to the end of the experiment (Figure 2 and Table 2).

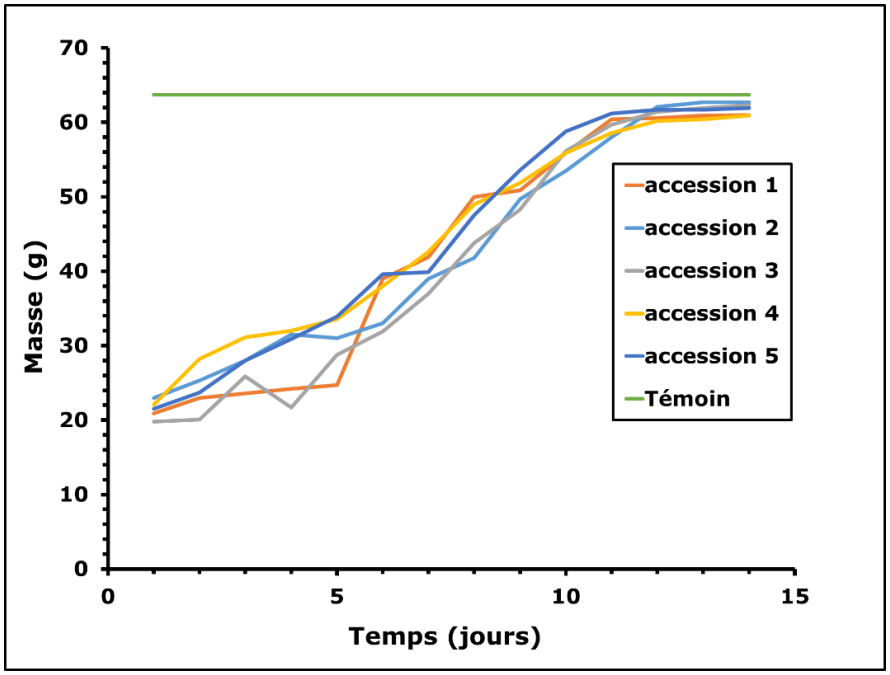

Figure 1. Curves showing the variation in mouse consumption over time.

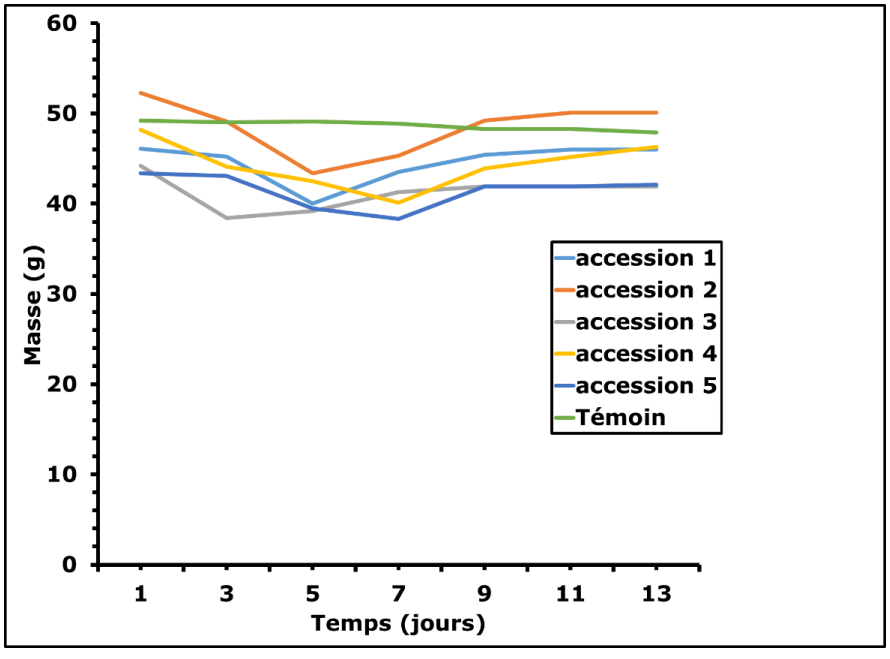

Figure 2. Curves of mouse weight variations over time.

Table 2. Evaluation of the urea parameter.

Batches of mice that were subjected to the mixed diet and standard diet experiments	Urea values obtained from different groups of mice that were subjected to the experiment for 14 days	Standard
Lot No. 1: DOGBO (144 mg/kg HCN)	1.018	0.15 to 0.45 g/L
Lot No. 2: ICRA 21 (135.1 mg/kg HCN)	0.931	
Lot No. 3: MAMBERE (134.66 mg/kg HCN)	0.809	
Lot No. 4: ICRA 51 (103.33 mg/kg HCN)	0.756	
Lot No. 5: ICRA 14 (100.66 mg/kg HCN)	0.639	
Lot No. 6: WITNESS	0.428	

The results clearly show that the urea content measured in mice receiving the mixed diet is as follows: Accession DOGBO = 1.018 g/L; Accession ICRA21 = 0.931 g/L; Accession MAMBERE = 0.809 g/L; Accession ICRA51 = 0.756 g/L; Accession ICRA14 = 0.639 g/L and the control batch = 0.42 g/L. All these values are higher than the standard (0.15 to 0.45 g/L) except in the case of the control. Thus, the comparison test of the DOGBO accession with the control gives this difference.

```
        Welch Two Sample t-test

data:  DOGBO and temoin
t = 99.244, df = 6.5221, p-value = 1.289e-11
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 0.5763815 0.6049519
sample estimates:
mean of x mean of y
1.0183333 0.4276667
```

The results show that the following values were obtained for creatinine: Accession DOGBO = 19.090 mg/L; Accession ICRA21 = 18.800 mg/L; Accession MAMBERE = 17.700 mg/L; Accession ICRA51 = 17.100 mg/L; Accession ICRA14 = 15.900 mg/L and the control batch = 10.200 mg/L. All these values are higher than the standard (6 to 14 mg/L), except in the case of the control. Thus, the comparison test between the accessions and the control gives:

>test (DOGBO, witness, conf.level = 0.95) Welch Two Sample t-test data: DOGBO and witness

t = 164.36, df = 9.9376, p-value < 2.2e−16. Alternative hypothesis: true difference in means is not equal to 0. 95 percent confidence interval: 9.573978, 9.837356; sample estimates: mean of x, mean of y: 19.86100, 10.15533.

>t.test (MAMBERE, witness, conf.level = 0.95) Welch Two Sample t-test data: MAMBERE and witness

t = 81.175, df = 7.6181, p-value = 1.811e−12. Alternative hypothesis: true difference in means is not equal to 0. 95 percent confidence interval: 6.729299, 7.126368; sample estimates: mean of x, mean of y: 17.08317, 10.15533.

>t.test (ICRA51, control, conf.level = 0.95) Welch Two Sample t-test data: ICRA51

and control

t = 37.524, df = 5.4103, p-value = 9.496e−08. Alternative hypothesis: true difference in means is not equal to 0. 95 percent confidence interval: 7.060687, 8.074313; sample estimates: mean of x, mean of y: 17.72283, 10.15533 (Table 3).

Table 3. Evaluation of the creatinine parameter.

Batches of mice that were subjected to the experiment with mite food and standard food	Creatinine values obtained from different groups of mice that were subjected to the experiment for 14 days	Standard
Lot No. 1: DOGBO (144 mg/kg HCN)	19.090	6 to 14 mg/L
Lot No. 2: ICRA 21 (135.1 mg/kg HCN)	18.800	
Lot No. 3: MAMBERE (134.66 mg/kg HCN)	17.700	
Lot No. 4: ICRA 51 (103.33 mg/kg HCN)	17.100	
Lot No. 5: ICRA 14 (100.66 mg/kg HCN)	15.900	
Lot No. 6: WITNESS	10.200	

Table 4. Evaluation of the ALAT parameter.

Batches of mice that were subjected to the mixed diet and standard diet experiment	ALAT values obtained from different groups of mice that were subjected to the experiment for 14 days	Standard
Lot No. 1: DOGBO (144 mg/kg HCN)	1374.833	4 to 40 IU/L
Lot No. 2: ICRA 21 (135.1 mg/kg HCN)	399.00	
Lot No. 3: MAMBERE (134.66 mg/kg HCN)	199.611	
Lot No. 4: ICRA 51 (103.33 mg/kg HCN)	160.167	
Lot No. 5: ICRA 14 (100.66 mg/kg HCN)	135.444	
Lot No. 6: WITNESS	12.906	

The results show regarding the ALAT parameter that the following values were obtained: Accession DOGBO = 1374.833 IU/L; Accession ICRA21 = 399.00 IU/L; Accession MAMBERE = 199.611 IU/L; Accession ICRA51 = 160.167 IU/L; Accession ICRA14 = 135.444 IU/L, and the control batch = 12.906 IU/L. All these values are higher than the standard (4 to 40 IU/L) except in the case of the control. Thus, the comparison test between the accessions and the control gives:

>t.test (DOGBO, witness, conf.level = 0.95) Welch Two-Sample t-test Data: DOGBO and witness

t = 17338, df = 6.1152, p-value < 2.2e−16. Alternative hypothesis: true difference in means is not equal to 0. 95 percent confidence interval: 1361.736, 1362.119; sample estimates: mean of x, mean of y: 1374.83350, 12.90567.

>t.test (MAMBERE, witness, conf.level = 0.95) Welch Two-Sample t-test data: MAMBERE and witness

t = 850.92, df = 5.1318, p-value = 2.055e−14. Alternative hypothesis: true differ-

ence in means is not equal to 0. 95 percent confidence interval: 186.1458, 187.2652; sample estimates: mean of x, mean of y: 199.61117, 12.90567.

>t.test (ICRA51, witness, conf.level = 0.95) Welch Two-Sample t-test data: ICRA51 and witness

t = 459.9, df = 5.0614, p-value = 6.821e−13. Alternative hypothesis: true difference in means is not equal to 0. 95 percent confidence interval: 146.4409, 148.0811; sample estimates: mean of x, mean of y: 160.16667, 12.90567 (Table 4).

The results show that the measured ASAT parameter, the following values were obtained: Accession DOGBO = 1451.389 IU/L; Accession ICRA21 = 500.556 IU/L; Accession MAMBERE = 270.611 IU/L; Accession ICRA51 = 242.000 IU/L; Accession ICRA14 = 174.389 IU/L; and the control batch = 17.950 IU/L. All these values are higher than the standard (4 to 38 IU/L), except in the case of the control. Thus, the comparison test between the accessions and the control gives:

>t.test (DOGBO, witness, conf.level = 0.95) Welch Two Sample t-test data: DOGBO and witness

t = 4824.3, df = 5.4446, p-value < 2.2e−16. Alternative hypothesis: true difference in means is not equal to 0. 95 percent confidence interval: 1432.694, 1434.184; sample estimates: mean of x, mean of y: 1451.389, 17.950.

>t.test (MAMBERE, witness, conf.level = 0.95) Welch Two Sample t-test Data: MAMBERE and witness

t = 626.85, df = 5.2371, p-value = 5.698e−14. Alternative hypothesis: true difference in means is not equal to 0. 95 percent confidence interval: 251.6392, 253.6835; sample estimates: mean of x, mean of y: 270.6113, 17.9500.

>t.test (ICRA51, control, conf.level = 0.95) Welch Two Sample t-test Data: ICRA51 and witness

t = 286.59, df = 5.062, p-value = 7.455e−12. Alternative hypothesis: true difference in means is not equal to 0. 95 percent confidence interval: 222.0479, 226.0524; sample estimates: mean of x, mean of y: 242.0002, 17.9500 (Table 5).

Table 5. Evaluation of the ASAT parameter.

Batches of mice that were subjected to the mixed diet and standard diet experiment	ASAT values obtained from different groups of mice that were subjected to the experiment for 14 days	Standard
Lot No. 1: DOGBO (144 mg/kg HCN)	1451.389	4 to 38 IU/L
Lot No. 2: ICRA 21 (135.1 mg/kg HCN)	500.556	
Lot No. 3: MAMBERE (134.66 mg/kg HCN)	270.611	
Lot No. 4: ICRA 51 (103.33 mg/kg HCN)	242.000	
Lot No. 5: ICRA 14 (100.66 mg/kg HCN)	174.389	
Lot No. 6: WITNESS	17.950	

3.2. Synthesis of Biochemical Parameters

3.2.1. Consumption and Body Weight

A significant decrease in weight was observed in the treatment groups compared

to the control group, suggesting an anorexigenic or metabolic effect.

3.2.2. Biochemical Parameters

Levels of urea, creatinine, ASAT, and ALAT were significantly elevated in animals that had been exposed to cassava leaf powder. Access to DOGBO revealed the most marked variations.

3.3. Statistical Analyses

Welch's t-tests validated the significant differences between the exposed samples and the reference group ($p < 0.05$).

3.4. Histological Observations

Histological observations of vital organs of mice fed mixed and standard diets across different batches.

Histological observations of the vital organs of mice fed a standard mixed diet.

1) Histological section of mice (organs) has received 100% of the standard food.
2) Histological section of mice (organs) has received 25% of the powdered leaves of the DOGBO accession +75% of the standard food.

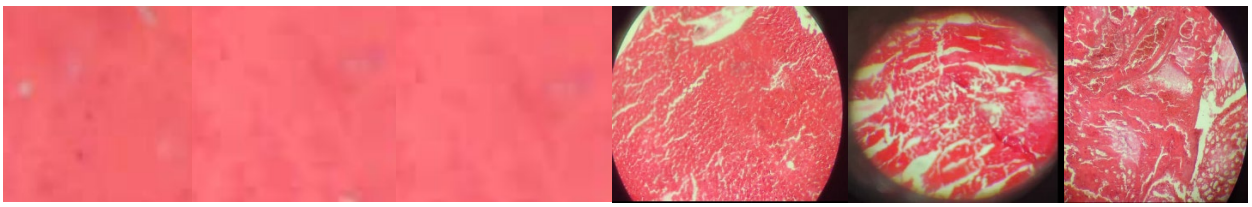

Figure 3. Histological section of the vital organs of mice.

The control receiving 100% of the standard diet has normal vital organs or normal tissues. On the other hand, the control receiving a mixed diet with various concentrations of hydrocyanic acid presents abnormalities in the vital organs in particular: the heart presents atrophy of the cardiac muscle cells with fibrosis and edema; the liver presents hepatocyte necrosis in several foci with fibrosis and infiltrate; and the kidneys present a focus of ischemic necrosis with persistence of glomerular silhouettes followed by edema (**Figure 3**).

3.5. Histological Observations Specific to the Vital Organs of Mice Receiving Mixed and Standard Food, Depending on the Batches

Control receiving 100% of the standard feed

All vital organs examined, including the Heart, Liver, and Kidneys of mice fed a standard diet, were normal, *i.e.*, the Heart had normal tissue, as did the Liver and Kidneys.

Control receiving mixed feed (powdered leaves of the accession DOGBO at a concentration of 144 mg/kg of HCN + standard food)

The vital organs of these mice examined present anomalies, in particular: the heart

presents atrophy of the cardiac muscle cells; the liver presents hepatocyte necrosis in several foci; and the kidneys present a focus of ischemic necrosis with persistence of glomerular silhouettes.

Control receiving food (powdered leaves of the accession ICRA 21 at a concentration of 135.1 mg/kg of HCN + the standard food)

The results show that all vital organs of the mice examined are abnormal, including: the heart shows atrophy of myocardial cells; the liver shows foci of hepatocyte necrosis; and the kidneys show ischemic necrosis of the cortex but no sign of tumor.

Control receiving mixed feed (powdered leaves of the accession MAMBERE 134.66 mg/kg of HCN + the food stand)

The vital organs of the mice observed present the following anomalies: the heart presents an atrophic-looking cardiac muscle; the liver presents congestion, notably hemorrhagic and black pigments; the kidneys present congestion.

Control receiving mixed feed (powdered leaves of the accession: ICRA 51 at a concentration of 103.33 mg/kg of HCN + the standard food)

These results show that all vital organs examined are abnormal, including: the heart presents cardiac tissue with fibrosis and edema; the liver presents hepatic tissue with fibrosis and infiltrate; and the kidneys present fragmented renal tissue with edema.

Control receiving mixed feed (powdered leaves of the accession ICRA 14 at a concentration of 100.66 mg/kg of HCN + standard food)

Visible changes in the vital organs examined show: the heart has congestion followed by necrosis; the liver has black pigments ++++; and the kidneys have necrosis plus a white spot.

4. Discussions

The growth of mice in the control group was statistically significant compared to the treated groups (GLM, $P < 0.0001$), which indicates that the diet provided more nutrients that could contribute to the acquisition of weight gain in mice after 14 days compared to the other treatments. The results also showed a decrease in the weight of mice whose diet was composed of 25% cassava leaves and 75% corn. The GLM model revealed a highly significant difference compared to the weights of mice treated only with corn (GLM, $P < 0.0001$). This explains why the diet composed of 25% cassava leaves can be recommended in the diet of mice.

The results obtained after the biochemical analyses were out of standard except for the control group. A very significant elevation of the hepatic and renal markers was noted in particular: the Urea parameter, for all the accessions evaluated, was out of standard (0.15 to 0.45 g/L): Accession DOGBO = 1.018; Accession ICRA21 = 0.931; Accession MAMBERE = 0.809; Accession ICRA51 = 0.756; Accession ICRA14 = 0.639 and the control group = 0.42. The creatinine parameter, for all the accessions evaluated, was out of standard (6 to 14 mg/L): Accession DOGBO = 19.090; Accession ICRA21 = 18.800; Accession MAMBERE = 17.700; Accession

ICRA51 = 17.100; Accession ICRA14 = 15.900 and the control batch = 10.200. The ALAT parameter, for all accessions evaluated, was out of standard (4 to 40 IU/L): Accession DOGBO = 1374.833; Accession ICRA21 = 399.000; Accession MAMBERE = 199.611; Accession ICRA51 = 160.167; Accession ICRA14 = 135.444 and the control batch = 12.906. The ASAT parameter, for all accessions evaluated, was out of standard (4 to 38 IU/L): Accession DOGBO = 1451.389; Accession ICRA21 = 500.556; Accession MAMBERE = 270.611; Accession ICRA51 = 242.000; Accession ICRA14 = 174.389 and the control group = 17.950. This was accompanied by weight loss and visible changes in the organs examined, highlighting the presence of atrophy, dystrophy, ischemia, and necrosis in the livers, kidneys, and hearts of the mice tested, except the control group. Cassava leaves contain cyanogenic substances that can be dangerous if not properly processed. The increase in liver enzymes and abnormalities in kidney functions observed indicate organ toxicity. These results support previous research regarding the hazards of cassava products. Cassava accessions such as DOGBO or ICRA21 show higher toxicity levels. Macroscopic examination of kidneys and livers of mice subjected to cassava leaf powders at different concentrations for batches L1 (144 mg/kg), L2 (134.66 mg/kg), L3 (135.1 mg/kg), L4 (103.33 mg/kg), L5 (100.66 mg/kg) reveals a specific or particular lesion compared to the same organs of mice in control batches. Some abnormalities are observed in the colon of mice exposed to cassava leaf powder: these are essentially nodules on the distal and proximal parts of the colon, more or less significant hemorrhages, and a narrowing of the colonic wall, particularly in the distal part of the colon. These abnormalities or lesions are found in all mice in the batches subjected to cassava leaf powders at the different concentrations used. Histological analysis in the control batch shows the histological organization of a normal liver. Concerning the histopathology of the liver of mice subjected to standard food. But on the other hand, for animals from different batches that were subjected to cassava leaf powders at different concentrations, specifically, the histological observation in general of these different batches of accessions shows the presence of clarified cell clusters with congestion, as the study highlighted above.

Monitoring of weight evolution showed the presence of significant weight loss in animals subjected to cassava leaf powders compared to controls. Similarly, other authors have also shown the effect of administering plant leaf powders on the body weight of animals [15] [16]. Weight change is used as a general indicator of the adverse effects of chemical compounds [17]. Thus, weight loss is correlated with the physiological state of the animal and can be explained not only by anorexia [18], but also by the alteration of the metabolism of the animals [19]. In our case, the animals in the batches subjected to cassava leaf powders all suffered dehydration, as evidenced by wet feces. The hematopoietic system is one of the most sensitive targets for toxic substances. It represents an important marker of the physiological and pathological state of humans and animals [19] [20]. Therefore, any change in hematological parameters has a predictive value for human poisoning

when the data are translated from animal studies [15] [21]. In the present study, an increase in biochemical parameters was reported. The elevation of transaminase levels in mice subjected to cassava leaf powders indicates poisoning [22]. The liver is the main site of detoxification of natural substances [23]. Therefore, a study of liver function may be useful in assessing the toxic effects of cassava leaf powders on the liver.

The results of the transaminase assays, ASAT or GOT, and ALAT or GPT, showed a very significant increase in GPT in the batches treated with cassava leaf powders. These results are similar to the work of [24]. Wistar rats exposed to 200 mg/L of cadmium sulfate have elevated transaminases. In the batches treated with the mixed feed ration, an increase in the level of GTP in the serum of the animals was observed. This increase in the level of GTP may be linked to a high level of vitamins. This also verifies the results of [25], who found that chronic alcohol consumption with Koutoukou (ACK) leads to a decrease in ASAT/ALAT levels in rats (Rattus norvegicus). According to our analyses, a high ASAT/ALAT level in the blood has an effect on the organs of the animals, and this generally indicates that the liver cells have been affected [14]. Transaminases (ALAT and ASAT) are the main enzymes for assessing the state of liver function [26]. In general, ASAT and ALAT are enzymes of mitochondrial and cytoplasmic origin. Indeed, any cell necrosis, destruction of the liver parenchyma, or an increase in the membrane permeability of hepatocytes leads to the flow of these enzymes into the bloodstream and therefore the increase of their serum levels [27]-[29]. Acute treatment of mice at a dose led to an increase in serum levels of transaminases (ASAT and ALAT). This increase is significant. Renal assessment is used to detect possible kidney dysfunction. It initially includes some biochemical tests: urea, creatinine [30]. Indeed, these parameters have high values in case of alteration of the renal filtration mechanism [31] [32]. Nature has an effect on high levels of urea and creatinine. The values of these biochemical parameters are elevated in the out-of-range intervals and have undergone large variations compared to controls. Furthermore, ALAT (Alanine aminotransferase) or SGPT (serum glutamylpyruvate transaminase) is a sensitive and specific marker of hepatocellular damage. An elevation of ALAT suggests liver disease except in cases of severe rhabdomyolysis or systemic myopathies. However, ALAT levels may be normal in cases of liver disease. Methotrexate-induced hepatitis and chronic hepatitis C may not be accompanied by an elevation of transaminases. However, chronic infection is most often characterized by fluctuating aminopeptidase values, which can sometimes be normal, sometimes elevated. Indeed, there is no correlation between the level of hepatocellular necrosis as objectified by biopsy and the level of transaminases. The degree of elevation of liver enzymes helps narrow down the differential diagnosis of a particular case: a mild (<2 - 3× the norm) to moderate (3 - 10× the norm) elevation is seen in alcoholic liver disease, steatosis, non-alcoholic steatohepatitis, and chronic viral hepatitis. In the face of a marked elevation (greater than 10× and especially 20× the norm), acute viral hepatitis, hepatic necrosis in-

duced by drugs or toxins, as well as hepatic ischemia, are mainly considered. Similarly, ASAT (aspartate aminotransferase) or SGOT (serum glutamyl oxaloacetate transferase) is less sensitive and less specific than ALAT for the liver. In case of ASAT/ALAT ratio > 1, muscle pathology must be excluded by measuring CK. In case of alcoholic liver disease, ASAT can be increased in isolation. An ASAT/ALAT ratio > 2 is found in 70% of alcoholic liver diseases, 26% of postnecrotic cirrhosis, 8% of chronic hepatitis, and 4% of acute viral hepatitis. The sensitivity of this ratio for alcohol-related damage is 70%, its specificity is 92%, and its positive and negative predictive values are around 80%. The ASAT/ALAT ratio can therefore help determine the probability that liver damage is due to alcohol, but if it is elevated, another cause should always be considered.

5. Conclusion

The research highlights the presence of short- and medium-term toxicity in the leaves of the cassava accessions evaluated. Appropriate treatment is crucial before the consumption of its by-products to avoid significant health risks. It is necessary to carry out strict genetic selection and raise public awareness before widespread distribution in food. The most toxic accessions should be effectively re-evaluated through the kinetic study of retting, including soaking, maceration, fermentation, drying at temperatures of 40˚C for a time, and storage, which can lead to a significant drop in the initial content of cyanogenic glucosides contained in cassava in order to guarantee their food safety.

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Conflicts of Interest

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

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