

Control of chemical contaminants and evaluation of oral subacute toxicity of an alcoholic polyherbal product “Plaie de ventre” marketed for health claims in Yopougon (Côte d’Ivoire).

Abstract

Objective: Alcoholic mixtures are highly appreciated by the Ivorian population to their low cost and for their health claims. Contaminants control and toxicity tests were carried out in order to check the harmlessness of extractable from polyherbal product “Plaie de ventre”.

Place and Duration of Study: The study was conducted at the Laboratory of Pharmacology and Clinical Pharmacy of Félix Houphouët-Boigny University for a period of 4 months.

Methods: The search for pesticide residues and metallic trace elements was carried out respectively by high-performance liquid phase chromatography and by atomic absorption with air-arc flame ethylene AAS 20, respectively. Subacute oral toxicity study involved daily administration of the extractable from the polyherbal product to three groups of rats for 28 days at doses of 21.06, 42.12, and 84.24 mg/kg bw.

Results: The detected residues of pesticides and trace metals in product “Plaie de ventre” mixture were consistent with the standards for their use. Moreover, the administration of extractable from this mixture did not result in any alteration of the body or relative weights of the rats, the structure of the kidney and liver at different doses. Finally, the analysis of the hematological and biochemical parameters indicated a noteworthy escalation ($p < 0.05$) in levels of white blood cells, blood platelets, and serum triglycerides.

Conclusion: The extract from the polyherbal “Plaie de ventre” caused changes in hematological and biochemical parameters compared to control group in treated rats. Consumers may face a long-term risk of developing hyperlipidemia.

Keywords: Polyherbal, Contaminants, Hematological, Biochemical.

1. INTRODUCTION

In Côte d’Ivoire, herbal medicinal products are highly developed with the aim of providing health care to the population [1]. These products could be found in various forms such as dry powders, plant organs, ointments, capsules, aqueous and alcoholic liquid products [2]. The latter forms are very popular in Ivorian bistros. Indeed, many people go to these traditional drinking places to consume alcoholic herbal concoctions whose health claims are attributed. Unfortunately, these alcoholic proposals contain a mixture of several plant species for which no safety data are available, and which could significantly alter the health of consumers in short-to-medium and long-term. Some cases of

intoxication have been reported in the press following the consumption of these alcoholic beverages. In 2015, cases of intoxication with 12 deaths were reported in Bocanda (Center of Côte d'Ivoire), in 2019, a similar intoxication with 08 deaths was described by a reporter in Abatta in the commune of Bingerville (South of Côte d'Ivoire), and in 2020, 09 deaths were reported in the commune of Abobo (South of Côte d'Ivoire)[3-4-5]. In addition, some people intoxicated by these products indicated some side effect such as: digestive disorders, dermatological disorders, neurological disorders, dizziness, headache, asthenia and hyperthermia [6]. One of the most popular alcoholic polyherbal products marketed in some communes of Abidjan (Côte d'Ivoire) is called "Plaie de Ventre". This product was widely used by consumers for its claims against stomach ache, abdominal pain, constipation and hemorrhoids [7]. It's a mixture of six plants including *Alchorneacordifolia*, *Piper guineense*, *Aframomum melegueta*, *Khaya senegalensis*, *Monodora myristica* and *Xylopi aethiopica*.

Although previous studies have shown that these plants taken individually are relatively non-toxic to humans [8-9-10], there is a little safety data available on this complex mixture.

Therefore, it's necessary to screen chemical control and harmlessness of the product "Plaie de Ventre" in order to ensure its safe use. Previously, oral acute toxicity studies conducted on this polyherbal product in Wistar rats showed that no behavioral changes in the rats, and estimated LD50 was greater than 5000 mg/kg bw. In addition, evaluation of hematological and biochemical parameters at 500 mg/kg bw revealed some changes in the values [7].

This study aims to provide further data on this product by analyzing chemical contaminants and performing a subacute oral toxicity test. This work therefore constitutes a food safety indicator to be disseminated to the scientific community, political and administrative authorities, bistro managers and consumers about the risks to which consumers of this alcoholic beverage are exposed, and to take steps to regulate the marketing of these drinks.

2. MATERIAL AND METHODS

2.1 Material

2.1.1 Polyherbal product "Plaie de Ventre".

The material was obtained from a traditional bistro owner in the town of Yopougon (Abidjan). It is a blend of a traditional alcohol (45°C) and six medicinal plants including *Alchorneacordifolia*, *Piper guineense*, *Aframomum melegueta*, *Khaya senegalensis*, *Monodora myristica* and *Xylopi aethiopica*.

2.1.2 Animal

Male and female Wistar rats of the species *Rattus norvegicus* eight (08) weeks old, with an average of 126.37 g body weight were used for the experiment. The rats were provided by the animal facility of Laboratory of Pharmacology and clinical Pharmacy, Faculty of Pharmaceutical and Biological Sciences, Félix Houphouët-Boigny University (Abidjan, Côte d'Ivoire).

2.2 Methods

2.2.1 Preparation of dry extract

A volume of 20 L of the polyherbal product “Plaie de ventre” was filtered through a square of cloth and then concentrated under reduced pressure at 40°C using a Heidolph G3 rotary evaporator. The extract was then dried in a Memmert type oven at 50°C to obtain the dry extractable of the alcoholic mixture and then stored in a jar for the experiments [11].

2.2.2 Heavy metals control

Heavy metals are present in the environment and can be absorbed by plants and accumulate in the roots, stems or leaves if the concentration increases in the soil. The most toxic of these are Arsenic (As), Cadmium (Cd), Mercury (Hg) and Lead (Pb). Atomic Absorption Spectrometry (AAS) is the method used for the determination of trace metals [12].

2.2.2.1 Procedure

A mass of 0.3 g of dry extract of the polyherbal product “Plaie de ventre” was calcined at 600°C for 5 hours in an oven until a white ash was obtained. After cooling, 5 mL of 1N nitric acid was added and evaporated to dryness on a sand bath (or hot plate). Five (5) mL of 1N hydrochloric acid was added to the residue and the whole is reheated at 400°C for 30 min. The calcined residue is removed from the furnace and 10 mL of 0.1N hydrochloric acid is added to the crucible to recover the product. The mixture obtained was poured directly into a 50 mL volumetric flask. The operation (washing the crucible with 10 mL of HCl at 0.1 mL) is repeated three times and the flask was filled to the mark. The supernatant was collected and filtered using 0.45 micron Whatman paper. The elements including Arsenic (As), Cadmium (Cd), Mercury (Hg) and Lead (Pb) in the solution are then determined by AAS [13].

2.2.2.2 Calculation of Maximum Admissible Concentrations

The permissible Daily Exposure (PDE) in µg/day is the maximum amount of an elemental impurity (IE) that can be absorbed by a 50 kg individual without causing adverse or toxic effects. It is from this PDE that the threshold specifications are determined, which make it possible to know whether the analyzed product contains a level of a given impurity that is compliant or not. Thus by definition, the Maximum Admissible Concentration (MAC) of an elemental impurity (EI) X is the maximum amount of that impurity that can be present (expressed in µg) per gram of finished product (drug) without causing health risks to the patient consuming that drug [14].

$$MAC = \frac{PDE (\mu\text{g/day})}{MDI \text{ of the alcoholic mixture (g/day)}}$$

With *MAC*: Maximum Admissible Concentration (µg/g)

PDE: Permissible Daily Exposure (µg/day)

MDI: Maximum Daily Dose of the Drug

2.2.2.3 Risk assessment

This step consists of comparing the analytical data from the previous step for each batch with the established Permissible Daily Exposure (PDE). In order to be able to compare each dosed concentration with the Permissible Daily Exposure (PDE) of the impurity element in question, it is necessary to determine threshold concentrations called specifications. The ICHQ3D guidelines specify two threshold specifications to be calculated:

- Threshold specification at 30% of the PDE.
- Threshold specification at 100% of the PDE.

So;

The 30% threshold is equal to; $0.3 \times \text{MAC}$ ($\mu\text{g/g}$)

And the threshold at 100% is equal to; $1 \times \text{MAC}$ ($\mu\text{g/g}$)

The introduction of a 30% threshold makes it possible to define control strategies. This is why the "control threshold" standard is used. According to ICHQ3D guidelines, if its trace metal concentration is below the 30% threshold [14].

2.2.3. Analysis of pesticide residues

The pesticides are phytosanitary products (herbicides, fungicides, insecticides) used for the protection of crops against predators and parasites [15]. The assay was carried out by a high performance liquid chromatography (SHIMADZU) type consisting of a TRAY tank, a DGU-20A5 degasser, a SIL-20A autosampler, an LC pump -20AT, a CTO-20A type oven and an SPD-20A UV/VIS detector. Data acquisition was performed using a computer equipped with LC Solution software.

2.2.3.1 Analytical protocol

2.2.3.1.1 Solid phase extraction

The Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction and purification method was used in this study [15]. Briefly, 2.5 g of sample was placed in an Erlenmeyer flask, 20 mL of dichloromethane was added, then homogenized on a shaker for 1 hour. The whole was filtered on Whatman paper in a ground bottom flask, evaporated to dryness with a BUTCHI type Rotavapor at 40°C . Then 5 mL of methanol was added to the flask, evaporated to dryness and the whole was transferred to a tube.

2.2.3.1.2 Purification

Activate the C18 cartridge with 10 mL methanol from 10 mL of acetonitrile. Pass the 10 mL of concentrated sample through the Rotavapor in the cartridge and allow the cartridge to dry for 30 minutes. Place a tubing under the cartridge. Dissolve the pesticides retained in the C18 cartridge by adding 10 mL of hexane. Transfer dropwise into the tube and measure the volume obtained. Then transfer to a vial for pesticide quantification using HPLC (SHIMADZU). Data acquisition was performed using a computer equipped with LC Solution software [16].

2.2.4. Subacute oral toxicity assessment method

The study was conducted according to OECD Guideline 407 and was performed on Wistar rat (preferred experimental species) [17]. Twenty-four albino Wistar rats were used and divided into four equal groups of three males and three females.

2.2.4.1. Conditioning of animals.

All animals were acclimated for two weeks to a temperature of $25 \pm 2^\circ\text{C}$ and alternating 12 hours of light and 12 hours of darkness with free access to food and water. Bedding was changed every three (3) days [18]. The diet consisted of IVOGRAIN® granules, which the rats had consumed continuously in the bottles [19].

2.2.4.2. Preparation and administration of doses.

The different concentrations used were prepared according to the concentration of extractable from the polyherbal product "Plaie de ventre" present in a 125 milliliter glass (glass used to consume the product) and administered according to the body weight of the animals. The decreasing concentrations were then used to highlight a relationship between the response and the dose administered. The difference between two doses was a factor of 2.

A total of 24 rats were individually caged and divided into four (4) batches of six (6) animals, including three (3) test batches (Batch 2 through 4) and one (1) control batch (Batch 1). Three extractable concentrations of 21.06, 42.12, and 84.24 mg/kg bw were tested in batches 2, 3, and 4, respectively, while batch 1 received only distilled water. The animals were fasted and allowed free access to water. They were weighed, and the test substance was administered daily by gavage (intraesophageal) at a rate of 1 milliliter (mL) per 100 g bw for 4 weeks. During the dosing period, animals were carefully observed daily for signs of toxicity. Rats were waterboarded and weighed every 7 days for 28.

2.2.4.3. Determination of some hematological and biochemical parameters

Rats were fasted the day before, weighed, anesthetized, and blood samples were collected at the beginning and end of the experiment (D0 and D28). Blood was collected in EDTA and dry tubes for the determination of hematological and biochemical parameters. These tests were performed using automated biochemical (Rayto RT-9200) and hematological (URIT-3000 Plus) analyzers. The influence of the administered dose on these various parameters was evaluated by complete blood count (CBC), determination of some hepatic markers (AST and ALT transaminases, alkaline phosphatase: ALP), kidney (creatinine and urea), lipids (total cholesterol, HDL, LDL and triglycerides) and carbohydrate metabolism (glycemia) [20].

For histopathological analysis, the livers and kidneys were isolated from the rats for each batch. These organs were rinsed with 0.9% saline, weighed and preserved in 10% formalin. The relative weight of each organ was calculated according to the formula below [21]. Histologic sections of these organs stained with hematoxylin-eosin were examined under the microscope.

$$Pr = (Po/Pa) \times 100$$



Pr: Relative weight of the organ (g/100 g)

*P*_o: Weight of the organ (g)

*P*_a: Body weight of the rat (g)

2.2.4.4. Statistical Analysis

GraphPad Prism software version 7.00 was used for statistical data analysis and graphical presentation. Data were analyzed by one-way ANOVA. Tukey's test was used to compare the means of the different extractable concentrations with the control batch. Results are presented as mean \pm standard error of the mean. The difference between two means was considered [22].

significant when $p < 0.05$ (*),

very significant when $p < 0.01$ (**)

highly significant when $p < 0.001$ (***)

and not significant when $p > 0.05$ (NS).

3. RESULTS

3.1 Trace metal analysis

The chemical analysis of the polyherbal product "Plaie de ventre" revealed the presence of several metallic trace elements including Arsenic (As), Cadmium (Cd), Mercury (Hg) and Lead (Pb). The presence of the trace metal contents is recorded in Table 1.

Table 1. Trace metals conformity

| Elément Metallic Trace | Mercury (Hg) | Lead (Pb) | Cadmium (Cd) | Arsenic (As) |
|---|--------------|------------|--------------|--------------|
| PDE: Permissible Daily Exposure ($\mu\text{g}/\text{day}$) | 30 | 5 | 5 | 15 |
| MDI: Maximum Daily Dose of the Drug (g/day) | 0.417 | 0.417 | 0.417 | 0.417 |
| MAC : Maximum Admissible Concentration ($\mu\text{g}/\text{g}$) | 71.94 | 11.99 | 11.99 | 35.97 |
| 30% Threshold Control Threshold) | 21.582 | 3.60 | 3.60 | 10.79 |
| 100% Threshold | 71.94 | 11.99 | 11.99 | 35.97 |
| Polyherbal concentrations in trace metals | 0.076 | -0.011 | -0.17 | 0.027 |
| Conclusion: Compliance with ICHQ3D guidelines | Compliance | Compliance | Compliance | Compliance |

3.2. Dosage of pesticide residues

The results of pesticide dosage are presented in Table 2.

The HPLC test revealed the presence of Propazine, Fenuron, Metazachlor, Aldicarb and Vinclozolin.

Table 2. Pesticide residues

| Family | Molécule | Concentrations (mg/kg) | LQ (mg/kg) |
|-------------------|-----------------------|-------------------------------|-------------------|
| TRIAZINE | Désisopropylatratzine | ND | 0.018 |
| | Désethylatrazine | ND | |
| | Simazine | ND | |
| | Cyanazine | ND | |
| | Atrazine | ND | |
| | Propazine | 0.003 | |
| | Terbutylazine | ND | |
| | Prometryn | ND | |
| | Terbutryn | ND | |
| TRIAZINONE | Métamitron | ND | 0.025 |
| | Hexazinone | ND | |
| | Metribuzin | ND | |
| DERIVES DE L'UREE | Fenuron | 0.040 | 0.018 |
| | Métoxuron | ND | |
| | Monuron | ND | |
| | Méthabenzthiazuron | ND | |
| | Chlortoluron | ND | |
| | Monolinuron | ND | |
| | Isoproturon | ND | |
| | Diuron | ND | |
| | Metobromuron | ND | |
| | Buturon | ND | |
| Linuron | ND | | |
| CHLOROACETAMIDE | Metazachlor | 0.034 | 0.010 |
| | Metolachlor | ND | |
| CARBAMATE | Aldicarb | 0.0043 | 0.009 |
| | Chlorpropham | ND | |
| ORGANOPHOSPHORE | Parathion-méthyl | ND | 0.009 |
| | Chlorfenvinphos | ND | |
| | Parathion-éthyl | ND | |
| DICARBOXIMIDES | Vinclozolin | 0.0012 | 0.009 |

ND: Not Detected; LQ: Limit of quantification (lowest measurable concentration).

3.3. Oral subacute toxicity

The effects associated with daily oral administration at repeated doses of the extractable were assessed after evaluation of behavioral parameters and weight growth, relative organ weights and biochemical parameters.

3.3.1. Clinical signs of intoxication

Behavioral observations throughout the study period revealed that no behavioral change was observed for 28 days.

3.3.2 Effect of extractable on body weight gain in rats

The evolution of body weight of the treated rats at the beginning (day 0) and at the end of the experiment (days 28) is shown in Figure 1. No significant changes were observed.

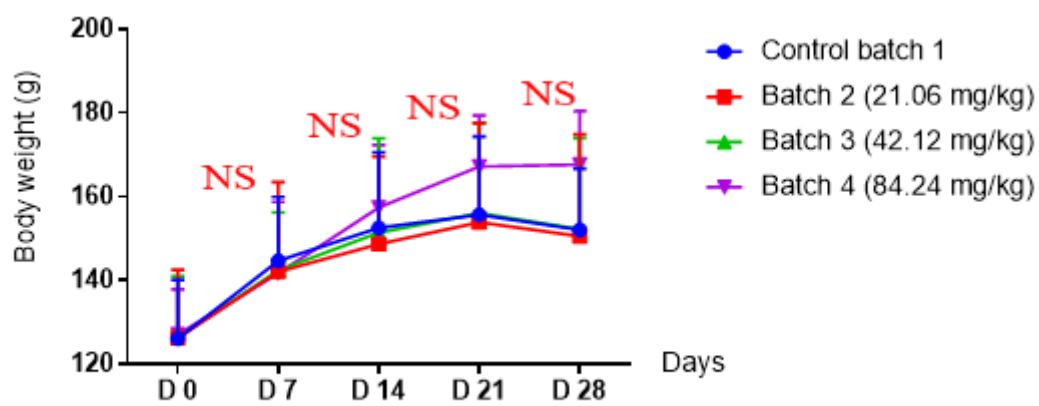


Fig. 1. Evolution of body weight during the 28-day experiment.

3.3.3 Effect of polyherbalextract “Plaie de Ventre” on variation of hematological

A significant increase in the number of white blood cells and platelets was observed at the doses of 42.12 mg/kg and 84.24 mg/kg, and even at the dose of 21.06 mg/kg for white blood cells (Figure 2 and 3). Figures 4 and 5 show the variation in red blood cells and hemoglobin concentration. In these graphs, no significant change in these parameters was observed at day 28 compared to the controls.

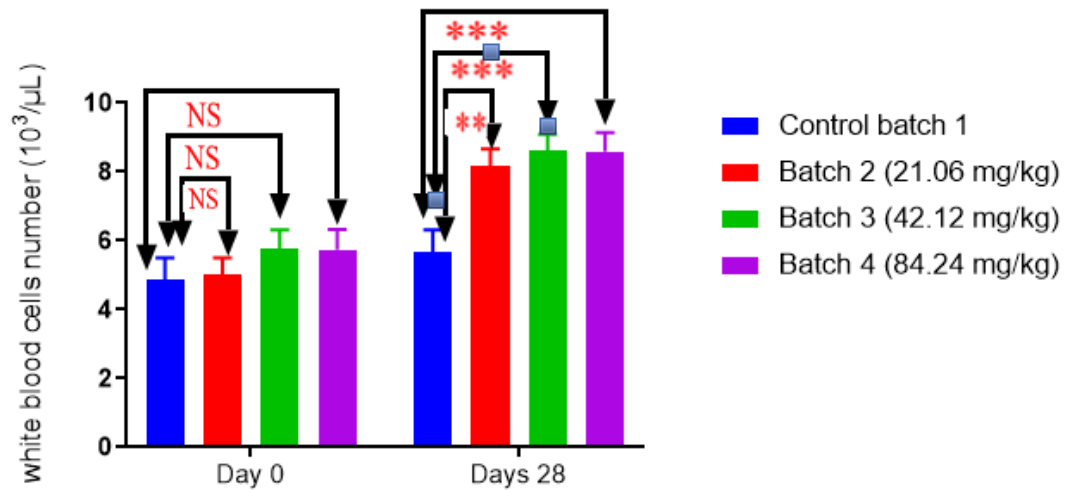


Fig. 2. Effect of polyherbal extract administration on the number of white blood

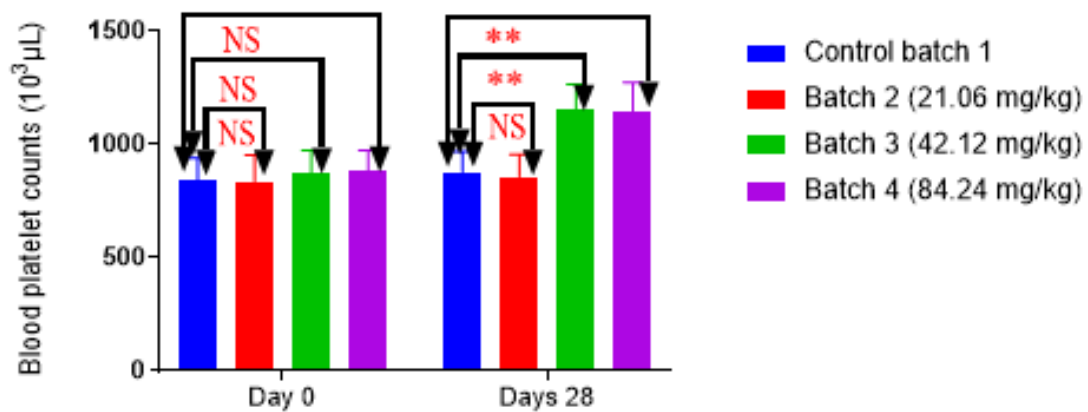


Fig. 3. Effect of polyherbal extract administration on the number of patelets

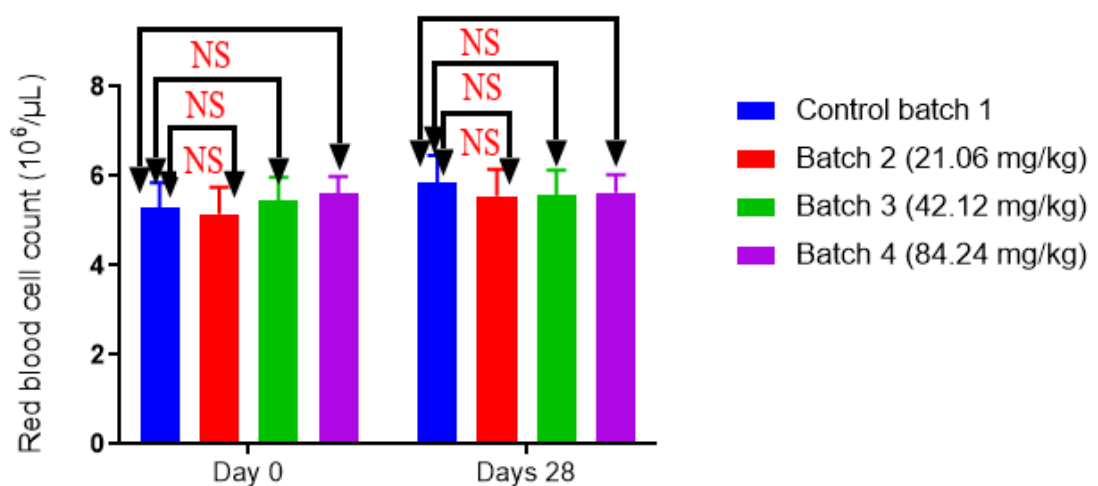


Fig. 4. Effect of polyherbal extract administration on the red blood cell count

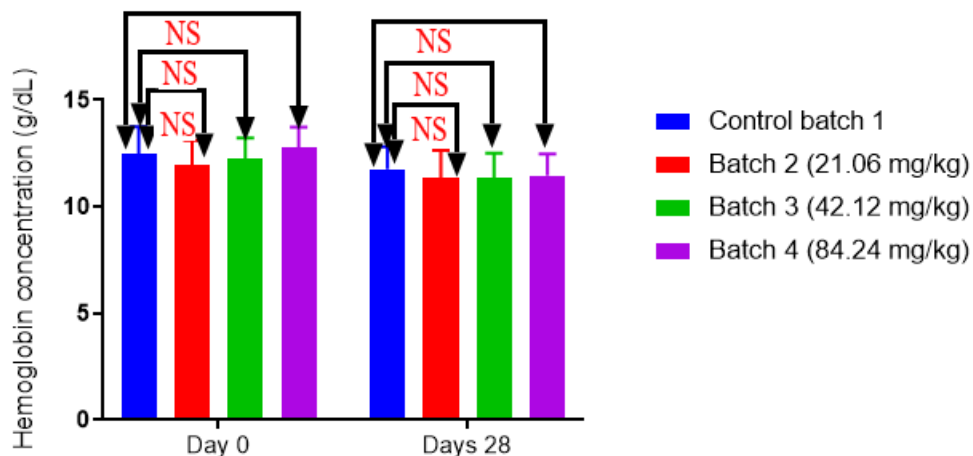


Fig. 5. Effect of polyherbal extract administration on hemoglobin concentration

3.3.4 Effects of polyherbal extract “Plaie de Ventre” on variation of biochemical parameters

The variations of biochemical parameters are shown in the Table 3.

Significant differences were observed between the batches of treated rats and the control batch for Triglycerol parameters while no difference was observed for kidney markers (Creatinemia and Urea), liver markers (Transaminase and Alkaline phosphatase), lipid parameters (Total cholesterol, HDL and LDL cholesterol) and blood glucose.

| Paramètres | Batch | Day 0 | Day 28 |
|---------------------------------|-----------------------|---------------|---------------|
| SGPT | Control batch 1 | 235.9 ± 8.47 | 243.8 ± 6.791 |
| | Batch 2 (21.06 mg/kg) | 227.3 ± 8.72 | 225.4 ± 6.03 |
| | Batch 3 (42.12 mg/kg) | 222.1 ± 7.93 | 226.6 ± 4.589 |
| | Batch 4 (84.24 mg/kg) | 227.3 ± 7.26 | 233,6 ± 5.13 |
| | | | |
| SGOT (AST) | Control batch 1 | 172.8 ± 5.494 | 86.72 ± 5.327 |
| | Batch 2 (21.06 mg/kg) | 161.7 ± 5.423 | 68.27 ± 6.02 |
| | Batch 3 (42.12 mg/kg) | 154.3 ± 5.253 | 82.15 ± 4.062 |
| | Batch 4 (84.24 mg/kg) | 152.8 ± 5.432 | 83.54 ± 4.86 |
| | | | |
| ALP | Control batch 1 | 144.2 ± 5.70 | 112.1 ± 3.55 |
| | Batch 2 (21.06 mg/kg) | 149.2 ± 5.50 | 121 ± 4,168 |
| | Batch 3 (42.12 mg/kg) | 158.8 ± 5.70 | 124.9 ± 3.248 |
| | Batch 4 (84.24 mg/kg) | 162.1 ± 5.74 | 123.9 ± 3.069 |
| | | | |
| Cholestérol Total | Control batch 1 | 0.532 ± 0.034 | 0.96 ± 0.06 |
| | Batch 2 (21.06 mg/kg) | 0.492 ± 0.048 | 1.03 ± 0.06 |
| | Batch 3 (42.12 mg/kg) | 0.455 ± 0.040 | 1.16 ± 0.08 |
| | Batch 4 (84.24 mg/kg) | 0.408 ± 0.040 | 1.16 ± 0.08 |
| | | | |
| HDL Cholestérol | Control batch 1 | 0.380 ± 0.021 | 0.24 ± 0.016 |
| | Batch 2 (21.06 mg/kg) | 0.350 ± 0.016 | 0.26 ± 0.016 |
| | Batch 3 (42.12 mg/kg) | 0.360 ± 0.021 | 0.29 ± 0.018 |
| | Batch 4 (84.24 mg/kg) | 0.340 ± 0.013 | 0.29 ± 0.028 |
| | | | |
| LDL Cholestérol | Control batch 1 | 0.152 ± 0.01 | 0.45 ± 0.03 |
| | Batch 2 (21.06 mg/kg) | 0.133 ± 0.01 | 0.46 ± 0.04 |
| | Batch 3 (42.12 mg/kg) | 0.145 ± 0.01 | 0.54 ± 0.03 |
| | Batch 4 (84.24 mg/kg) | 0.147 ± 0.01 | 0.54 ± 0.03 |
| | | | |
| Triglycerol | Control batch 1 | 1.47 ± 0.060 | 1.38 ± 0.05 |
| | Batch 2 (21.06 mg/kg) | 1.34 ± 0.067 | 1.54 ± 0.06 |
| | Batch 3 (42.12 mg/kg) | 1.30 ± 0.061 | 1.53 ± 0.08 |
| | Batch 4 (84.24 mg/kg) | 1.32 ± 0.060 | 1.65 ± 0.07* |
| | | | |
| Créatinine | Control batch 1 | 5.08 ± 0.08 | 3.10 ± 0.2082 |
| | Batch 2 (21.06 mg/kg) | 5.17 ± 0.10 | 3.02 ± 0.1869 |
| | Batch 3 (42.12 mg/kg) | 5.17 ± 0.10 | 2.70 ± 0.1693 |
| | Batch 4 (84.24 mg/kg) | 5.25 ± 0.11 | 2.53 ± 0.2092 |
| | | | |
| Urea | Control batch 1 | 0.127 ± 0.002 | 0.077 ± 0.005 |
| | Batch 2 (21.06 mg/kg) | 0.129 ± 0.002 | 0.075 ± 0.004 |
| | Batch 3 (42.12 mg/kg) | 0.129 ± 0.002 | 0.067 ± 0.005 |
| | Batch 4 (84.24 mg/kg) | 0.131 ± 0.003 | 0.063 ± 0.005 |
| | | | |
| Glycemia (Blood glucose) | Control batch 1 | 0.86 ± 0.05 | 1.20 ± 0.057 |
| | Batch 2 (21.06 mg/kg) | 0.84 ± 0.04 | 1.17 ± 0.054 |
| | Batch 3 (42.12 mg/kg) | 0.85 ± 0.04 | 1.25 ± 0.031 |
| | Batch 4 (84.24 mg/kg) | 0.84 ± 0.04 | 1.26 ± 0.036 |
| | | | |

Table 3: Variations biochemical parameters on Day 0 and Days 28

* : Significant difference, ** : Very significant difference *** : Highly significant difference. SGPT (Sérum Glutamic Pyruvic Transférase), SGOT (Sérum Glutamic oxaloacetic Transférase), HDL (.High Density Lipoproteins), LDL (Low Density Lipoprotéins), APL (Alkaline Phosphatase).

3.3.5. Effects of the polyherbal product "Plaie de ventre" on relative organ weights

The relative weights of the organs are presented in Table 4.

No significant difference was observed between the control and treated batches for these parameters.

| Batches | Relative kidney weights | Relative liver weights |
|------------------------------|-------------------------|------------------------|
| Batch 1 (Control) | 0.7204 ± 0.045 | 4.567 ±0.123 |
| Batch 2 (21.06 mg/kg) | 0.7752 ± 0.116 | 4.141 ± 0.436 |
| Batch 3 (42.12 mg/kg) | 0.7034 ± 0.018 | 3.711 ± 0.106 |
| Batch 4 (84.24 mg/kg) | 0.6936 ± 0.026 | 3.899 ±0.132 |

Table 4. Effect of the administration of extractable on the relative weight of the organs

The results are expressed as mean ± standard deviation (n=3).

3.3.5.1. Effect of extractable on hepatic and renal tissues

Analysis of the histological sections did not reveal any change in the structure of the liver and kidney of the rats. However, hyperchromatosis was observed in the structure of the liver (Figure 6).

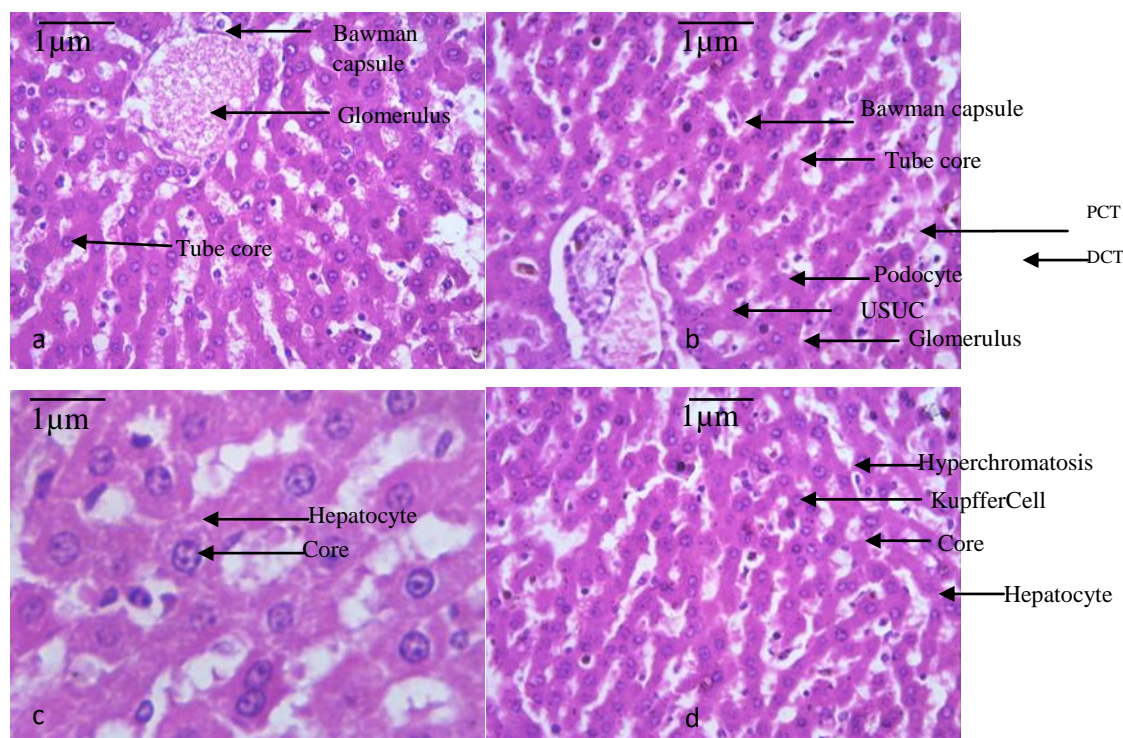


Fig. 6. Histological section of kidney (a, b) and liver(c, d)

a: View of a kidney section taken from the rats of the control batch, Gx400,

b: View of a kidney section taken from rats treated at the dose of 84.24 mg/kg de bw, Gx400,

PCT: Proximal Convolved Tube, DCT: Distal Convolved Tube, USUC:Urinaryspace or urinarychamber.

c: View of liver section taken from the control batch, Gx400,

d: View of a liver section taken from rats treated at the dose of 84.24 mg/kg bw, Gx400 Hematoxylin – eosin staining.

4. DISCUSSION

The aim of this study was to evaluate the safety of the extractable mixture of “Plaie de ventre” after daily oral administration for 28 days on the biochemical, hematological and histological parameters in Wistar rats.

The dosage of trace metals revealed the presence of Mercury, Cadmium, Lead, and Arsenic. Our results are in conformity with those of [12]; who noted the presence of Lead, Cadmium and Arsenic in the stems and roots of *Zygodium*. The solvent used, the soil conditions, the non-compliance with good manufacturing practices for the mixture and the solvent could explain this contamination. Furthermore, a study carried out by Koffi et al. [23] on the quality of traditional alcohol “Koutoukou” in relation to the different traditional manufacturing processes in the main production areas in Côte d'Ivoire showed contamination of “Koutoukou” with Lead and Cadmium. According to these authors, this contamination is due to the lack of training of producers based on Good Manufacturing Practices (GMP). Trace metals are naturally present in soil, water and air. The most toxic among are cadmium, arsenic, lead and mercury [24]. According to [25], toxic metals, even at low concentrations, have a polluting nature with harmful effects on living organisms. This assertion is supported by the work of [26] on female rats. In these studies, an increase in the level of white blood cells was observed after oral administration of lead acetate at a rate of 0.2% in double-distilled water from the first day of gestation until weaning. Despite the conformity of the residues present in the mixture of this study, it could affect the health of consumers.

The presence of pesticide residues (Propazine, Fenuron, Metazochor, Aldicarb and Vinclozolin) in the mixture could be explained by their use for the cultivation of plants, the chemical pollution of the environment and also by the non-respect of the agricultural practices (use of the minimum quantities necessary to effectively protect the crops).

A pesticide residue is the portion of a chemical or its degradation product that remains on the plant, in parts of the plant or in the soil [27]. Maximum residue limits (MRLs) in food are established on the basis of toxicological and agronomic data. They reflect good agricultural practices that result in residue levels that do not cause health effects [28]. According to the J.O.R.A.D.P. No. 13 of 03/09/2014, the limit of water quality parameters for human consumption for total pesticides is 0.5 µg/L [29]. This value is significantly higher than that found in the polyherbal product “Plaie de ventre” (0.0813 µg/L).

Therefore, the level of pesticide present in the polyherbal extract has no effect on the consumer.

Monitoring of the body weight of the treated rats showed no disturbance in the weight gain of the animals at the doses of 21.06, 42.12 and 84.24 mg/kg bw compared to the control. Thus, the extract does not affect the body-weight gain of the animals. These data suggest that the extractable of the mixture does not significantly affect body weight. These results do not support those of [30] who observed weight loss in males and females at the end of their experiment with extracts of *Aframomum melegueta*, *Xylopiiaethiopica* and *Khayasenegalensis*.

Regarding the effect of the alcoholic mixture on the hematological parameters, no significant modification of the rate of erythrocytes and hemoglobin was observed. Our results are in agreement with those of [30], who also did not observe any change in the level of erythrocytes and hemoglobin after administration of hydroethanolic extracts of *Aframomum melegueta* and *Kaya senegalensis* at a dose of 300 mg/kg bw in female rats. These results could lead to say that the extract of the studied mixture would not induce anemia. On the other hand, an increase in the level of white blood cells and platelets was observed in rats treated at all doses for leukocytes and at doses of 42.6; 84.24 for platelets. These results are consistent with those [30]. These authors also observed an increase in the level of white blood cells and platelets after 28 days of treatment with the aqueous extract of *Aframomum melegueta* and *Kaya senegalensis* at a dose of 300 mg/kg bw. The same observation was made at the level of white blood cells by Kplé et al. [31] on an herbal remedy of *Zanthoxylum leprieurii*, *Xylopiiaethiopica*, and *Harungaramadagascariensis*. The increase observed in this study would be due to the presence of bioactive substances capable of enhancing the immune response by increasing the level of white blood cells [32-33]. Indeed, white blood cells have the role of protecting and defending the body against bacteria, foreign substances, viruses, parasites, toxins and tumor cells. An increase in leukocytes may indicate activation of the immune system in response to infection, inflammation, or even necrosis [34]. The studies carried out by Aboli et al [7] revealed the presence of flavonoid and phenolic compound in the alcoholic polyherbal. According to these authors, these compounds have a strong antioxidant potential capable of activating the immune system. The presence of chemical these compound in the extract of this study may partially justify this claim.

Regarding the biochemical parameters, a significant increase in the serum level of triglycerides was observed at the dose of 84.24 mg/kg bw. Our results confirm those [35]. They showed a significant non-dose-related increase in triglycerides after administration of an ethanolic extract of *Alchornea cordifolia* leaves to rats at doses of 400 and 800 mg/kg bw. Indeed, the increase in triglycerides suggests that the mixture has the potential to induce hyperlipidemia, which is indicative of a lipid metabolism disorder [36].

Hypertriglyceridaemia is therefore a less potent cardiovascular risk factor than an increase in LDL cholesterol. Triglycerides are produced by the liver, but are also absorbed by the diet (sugars, alcohol) and constitute the body's largest stock of fatty acids. They are mainly found in adipose tissue. Their increase can be explained by a multifactorial origin combining a genetic predisposition and behaviour that favours them, such as excess weight, prolonged dieting, physical exercise, unbalanced diet and stress [37-38].

In contrast to renal (urea, creatinine), hepatic (AST, ALT and PAL) and certain lipid markers (total cholesterol, HDL and LDL), no significant changes were observed. Urea and creatinine are excellent markers of renal function, and changes in these reflect renal dysfunction. Transaminases and alkaline phosphatases are markers of liver function; an increase or decrease reflects liver dysfunction [35-36]. The lack of change in renal and hepatic parameters in treated rats suggests that the extract did not cause any renal or hepatic damage as supported by biochemical results.

5. CONCLUSION

This safety study of the polyherbal product "Plaie de ventre" showed the presence of trace metals and pesticide residues in accordance with the standards for use. In addition, repeated oral administration of this product for 28 days at doses of 21.06, 42.12 and 84.24 mg/kg bw did not affect body weight or the relative weights of the organs examined. On the other hand, the extract caused an increase in white blood cells and platelets. The alcoholic extract of this mixture had no adverse effect on biochemical parameters of liver and kidney function. However, a significant increase in triglycerides was observed on day 28 in rats of the groups treated at the dose of 84.24 mg/kg bw.

Prolonged consumption of this mixture could lead to a risk of toxicity on lipid parameters, consumers would be exposed to a risk of hyperlipidemia.

In view of the above, it is imperative to carry out more in-depth pharmacological studies to find the origin of the increase in leukocytes and platelets and to verify the ethnomedicinal use of the mixture.

ETHICAL APPROVAL

Ethical approval was gotten from the ethics committee from Jean Lorougnon guéde de daloa (Côte d'Ivoire) for animal experimentation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

Authors' contributions

This work was carried out in collaboration with the authors mentioned above. The author Aboli carried out the experiment and wrote the manuscript while the author Kporou Kouassi designed the methodology and contributed to the writing of the manuscript. The author Gbogbo moussa supervised the experiments. Finally, the authors Beugre Avit, Kouakou Gisèle and Djaman Allico corrected the manuscript.

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