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).

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Abstract

Introduction

Objective: Alcoholic mixtures are highly appreciated by the Ivorian population thanks to theirlowcost and for their health claims. Contaminants control and toxicity tests werecarried out in order to check the harmlessness of extractablefrompolyherbalproduct“Plaie de ventre”.

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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 extractablefrom the polyherbalproduct to three groups of rats for 28 days at doses of 21.06, 42.12, and 84.24 mg/kg bw.

Throughout the treatment, observations were made on animals and subsequently, hematological and biological parameters were evaluated along with histological examination.

Results: The detectedresidues 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 same observation was made when evaluating the structure of the kidney and liver at different doses. Finally, theanalysis 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 extractfrom the polyherbal“Plaie de ventre” caused changes in hematological and biochemicalparameterscompared 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

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popular in Ivorian bistros. Indeed, many people go to these traditional drinking places to consume alcoholic herbal concoctions which health claims are attributed. Unfortunately, these alcoholic proposals are 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 described the following signs: digestive disorders, dermatological disorders, neurological disorders, dizziness, headache, asthenia and hyperthermia [6].One 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*, *Piperguineense*, *Aframomummelegueta*, *Khayasenegalensis*, *Monodoramyristica*and *Xylopiaaethiopica*.

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.

2. MATERIAL AND METHODS

2.1 Material

2.1.1Polyherbalproduct"Plaie de Ventre".

The material was obtained from a traditional bistro owner in the town of Yopougon (Abidjan). It is a blend of a traditionalalcohol (45°C) and six medicinal plants including*Alchorneacordifolia, Piperguineense, Aframomummelegueta, Khayasenegalensis, Monodoramyristica and Xylopiaaethiopica.*

2.1.2 Animal

Male and femaleWistar rats of the species*Rattusnorvegicus*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).

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2.2Methods

2.2.1 Preparation of dry extract

A volume of 20 L of the polyherbalproduct “Plaie de ventre” wasfilteredthrough a square of cloth and then concentrated under reduced pressure at 40°C using a Heidolph G3 rotary evaporator. The extractwasthendried 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.(???)

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2.2.2 Heavy metals control

Atomic Absorption Spectrometry(S.A.A)is the method used for the determination of trace metals.

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2.2.2.1 Procedure

A mass of 0.3 g of dry extract of the polyherbalproduct “Plaie de ventre” wascalcined 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 hydrochloricacidisadded 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 cruciblewith 10 mL of HCl at 0.1 mL) isrepeatedthree times and the flaskisfilled to the mark. The supernatantiscollected and filteredthrough 0.45 micron Whatmanpaper. The elements including Arsenic (As), Cadmium (Cd), Mercury (Hg) and Lead (Pb) in the solution are then determined by AAS [11-12].

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2.2.2.2 Calculation of MaximumAdmissible 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 [13].

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

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 PDE. In order be able to compare each dosed concentration with the 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 x MAC (µg/g)

And the threshold at 100% is equal to; 1 x MAC (µg/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 [13].

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2.2.3. Analysis of pesticide residues

Pesticide dosage 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 QuEChERS extraction and purification method was used in this study [14]. 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 wholewasfiltered on Whatmanpaper 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.

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2.2.3.1.2 Purification

Activate the C18 cartridge with 10 mL methanol from 10 mL of acetonitrile. Pass the 10 mL of concentratedsamplethrough 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.[????]

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2.2.4. Subacute oral toxicity assessment method

The study was conducted according to OECD Guideline 407 and wasperformed on Wistar rat (preferredexperimentalspecies) [15]. Twenty-four albinoWistar rats wereused 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 ± 2°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 [16]. The diet consisted of IVOGRAIN® granules, which the rats had consumed continuously in the bottles [17].

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2.2.4.2. Preparation and administration of doses.

The different concentrations usedwerepreparedaccording to the concentration of extractablefrom the polyherbalproduct“Plaie de ventre”present in a 125 milliliter glass (glass used to consume the product) and administeredaccording 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 bwweretested 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 wasadministereddaily by gavage (intraesophageal) at arate 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 werewaterboarded and weighedevery 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 wereperformedusingautomatedbiochemical (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). [????]

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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 [18]. 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)

Po: Weight of the organ (g)

Pa: 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 ± standard error of the mean. The difference between two means was considered [???]

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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 chemicalanalysis of the polyherbalproduct“Plaie de ventre”revealed the presence of several metallic trace elements including Arsenic (As), Cadmium (Cd), Mercury (Hg) and Lead (Pb). The congruence of the trace metal contents is recorded in Table 1.

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Table 1. Trace metals conformity

Elément Metallic Trace	Mercury (Hg)	Lead (Pb)	Cadmium (Cd)	Arsenic (As)
PDE: Permissible Daily Exposure (µg/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 (µg/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

Familly	Molecule	Concentrations (mg/kg)	LQ (mg/kg)
TRIAZINE	Désisopropylatratzine	ND	0.018
	Désethylatrazine	ND	
	Simazine	ND	
	Cyanazine	ND	

	Atrazine	ND	
	Propazine	**0.003**	
	Terbuthylazine	ND	
	Prometryn	ND	
	Terbutryn	ND	
	Métamitron	ND	
TRIAZINONE	Hexazinone	ND	0.025
	Metribuzin	ND	
	Fenuron	**0.040**	
	Métoxuron	ND	
	Monuron	ND	
	Méthabenzthiazuron	ND	
	Chlortoluron	ND	
DERIVES DE L'UREE	Monolinuron	ND	0.018
	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	
	Parathion-méthyl	ND	
ORGANOPHOSPHORE	Chlorfenvinphos	ND	0.009
	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.

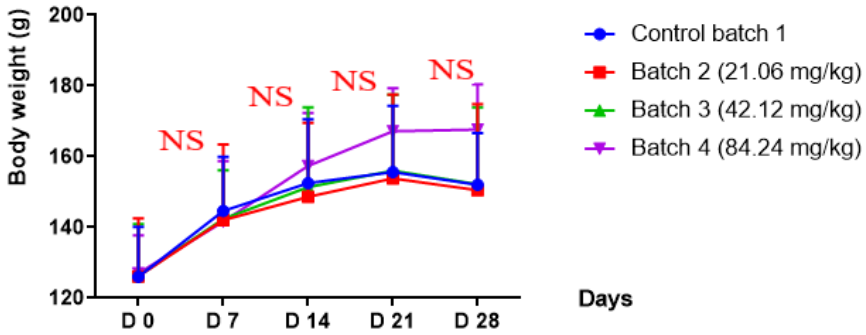

Fig. 1. Evolution of body weight during the 28-day experiment

4.3.3 Effect of polyherbalextract " Plaie de Ventre" on variation of hematological parameters

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.

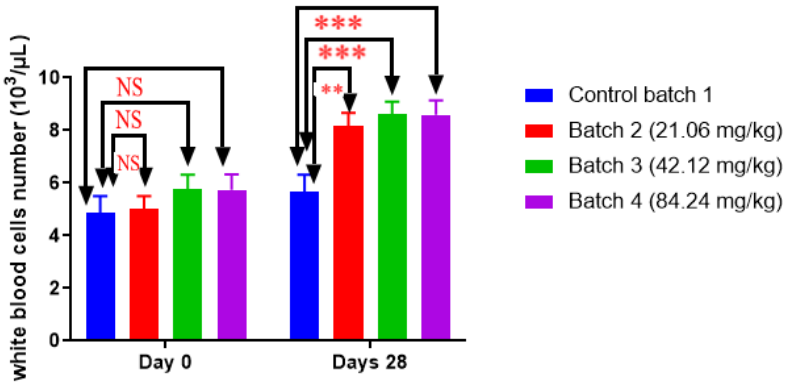

Fig. 2. Effect of polyherbal extract administration on the number of white blood

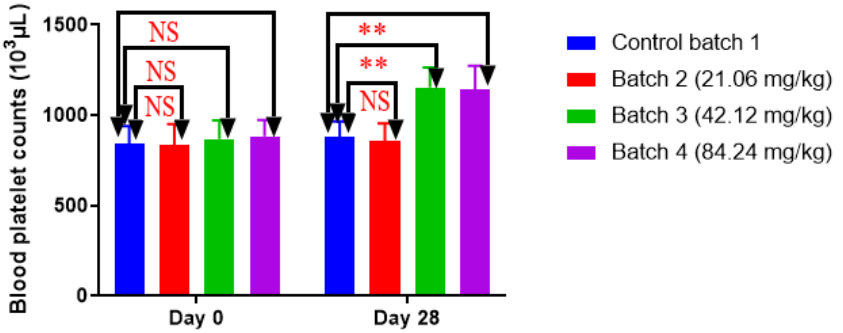

Fig. 3. Effect of polyherbal extract administration on the number of platelets

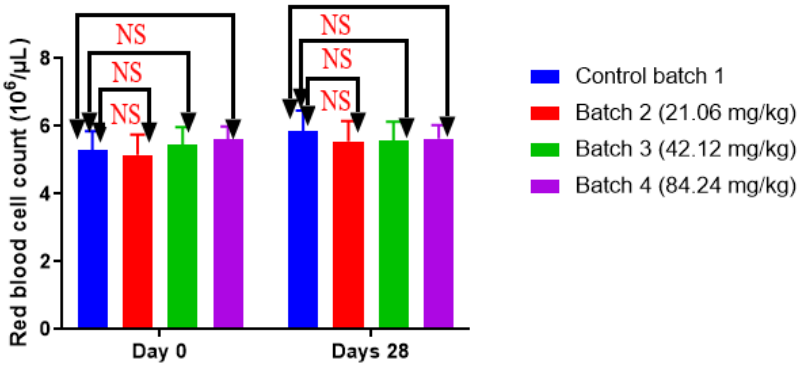

Fig.4. Effect of polyherbal extract administration on the red blood cell count

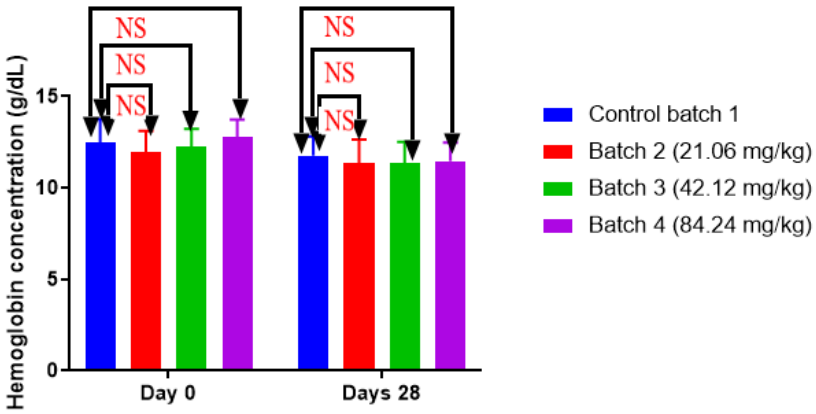

Fig. 5. Effect of polyherbal extract administration on hemoglobin concentration

3.3.4 Effects of polyherbalextract “Plaie de Ventre” on variation of biochemicalparameters

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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	Atch	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 oxaloaceticTransférase), HDL (.Hight Density Lipoproteins), LDL (Low Density Lipoprotéins), APL (Alkaline Phosphatase).

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3.3.4.1. 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.

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

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

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

3.3.4.2. 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).

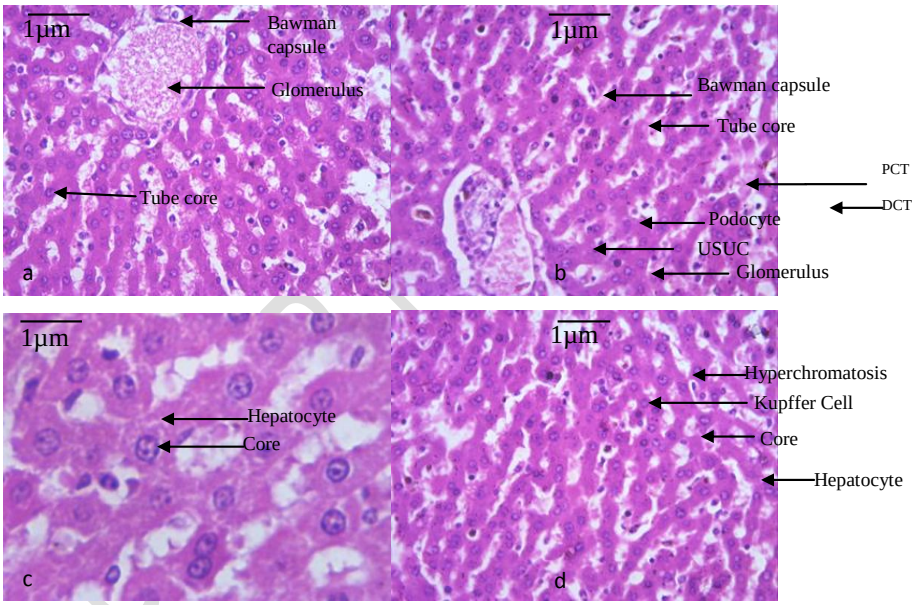

Fig. 6. Histological section of kidney (a, b) and liver(c, d)

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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 Convoluted Tube, DCT: Distal Convoluted Tube, USUC: Urinary space or urinary chamber.

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 thisstudywas to evaluate the safety of the extractablefrom the mixture of “Plaie de ventre”after daily oral administration for 28 days on the biochemical, hematological and histologicalparameters 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 [20]; whonoted the presence of Lead, Cadmim and Arsenic in the stems and roots of Zygophyllum. 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 [19] on the quality of traditionalalcohol "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 Cadmim. 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, [20]. According to [21], toxic metals, even at low concentrations, have a polluting nature with harmful effects on living organisms. This assertion is supported by the work of [22] 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 [23]. 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 [24]. 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 [25]. 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 [26] who observed weight loss in males and females at the end of their experiment with extracts of *Aframomummelegueta*, *Xylopiaaethiopica* 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 [26], who also did not observe any change in the level of erythrocytes and

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hemoglobinafter administration of hydroethanolicextracts of *Aframomummelegueta* and *Kayasenegalensis* 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 [26].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 *Aframomummelegueta* and *KayaSenegalensis* at a dose of 300 mg/kg bw. The same observation was made at the level of white blood cells by [27] on an herbal remedy of *Zanthoxylumleprieurii*, *Xylopiaaethiopica*, 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 [28-29]. 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 [30-31]. The presence of trace metals 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 [32]. They showed a significant non-dose-related increase in triglycerides after administration of an ethanolic extract of *Alchorneacordifolia* 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 [33].

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, creatinine, transaminase and alkaline phosphatase are markers of renal and hepatic function, respectively, their increase or decrease reflects dysfunction of these functions [32, 33]. 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.

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