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Original Article - Pharmacological Study

In vivo acute and subacute toxicity assessments of Viroscope®, a polyherbal ethanolic formulation produced in Lomé, Togo

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ABSTRACT

Background: Phyto-medicines have been used to treat various diseases since ancient times. However, several studies have reported the toxicity of some medicinal plant preparations. **Objective:** This study aimed to evaluate the *in vivo* acute and subacute toxicity of an ethanolic extract of medicinal plants, "VIROSCOPE®", produced by Viroscope SARL-U in Lomé, Togo. **Materials and Methods:** Acute toxicity assessment was conducted according to the "OECD guideline 423 for the assessment of the acute toxicity of chemicals." This study focused on the ready-to-use VIROSCOPE® and on the concentrated extract obtained after evaporation. Subacute toxicity was assessed according to the "OECD guideline 407 for the study of subacute toxicity of chemicals." Control rats; VIROSCOPE®; solvents; 100, 500, and 1000 mg/kg of the dry extract of VIROSCOPE® dissolved in drinking water were tested.The mortality rate, toxicity symptoms, food and water consumption, and changes in body weight were monitored. Biochemical and haematological parameters were also measured. Furthermore, histopathological analysis of hepatic and renal cells was conducted. **Results :** The median lethal dose (LD50) was > 5000 mg/kg. Repeated doses of the trial drug over a long period of 28 days had no major risk. In addition, it did not induce any pathological variations in biochemical and haematological parameters. According to histopathological results, there was no significant harmful effect to liver and kidney cells. **Conclusion:** VIROSCOPE® can be considered safe and must be administered strictly according to the indicated dosages on its bottle.

KEYWORDS: *VIROSCOPE®, Phytotherapy, acute toxicity, subacute toxicity*

INTRODUCTION

Phyto-medicines have been used to treat various diseases since ancient times, in the ancient empires of antiquity and the Middle Ages.[1,2] Nowadays, medicinal herbs remain effective against diseases affecting humans and are often used to discover new medically active ingredients.[3–5]

Phyto-medicines contain phyto-complexes, which are complexes of active molecules with specific biological activity. The biological activity of a phyto-complex is generally stronger than the sum of the activities of the individual active molecules, and the presence of substances with no specific activity can have a significant synergistic effect.[6,7]

Herbal medicines have been in great demand in recent years, with evidence of increased research on effective alternative therapies for the treatment of COVID-19. [8–10] In Africa, knowledge of herbal medicines is often taught orally and is transmitted to subsequent generations. Every traditional herbal medicine must undergo rigorous quality control and toxicity assays to ensure safety[11]. Indeed, in addition to the therapeutically active ingredients, medicinal plants can also contain harmful substances[12–15], often associated with dysfunction of several human vital organs, such as the heart, liver, and kidneys[16–18].

Thus, this study aimed to evaluate the *in vivo* acute and subacute toxicity of the phytomedicine "VIROSCOPE®" produced by Viroscope SARL-U in Lomé, Togo.

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PRESENTATION OF VIROSCOPE®

VIROSCOPE® is an ethanolic extract of medicinal plants produced by Viroscope SARL-U, Lomé, Togo. It is a dark yellow liquid with medium consistency and sweet and sour odour. It is used as an anti-inflammatory, antioxidant, anti-tumour, vaso protectant, booster of immune cell activity, and for the treatment of neuralgia and recurrent bacterial and mucosal oral and skin wounds. It is an oral liquid dosage form, with a total alcohol content of 30%.

To obtain VIROSCOPE®, (42% *Sodabi*) is used as solvent, to extract and preserve the active ingredients from the roots, hulls, and leaves of the medicinal plants. The other key ingredients of VIROSCOPE® will not be listed because of commercial confidentiality. *Sodabi* is a traditional distilled palm wine prepared in West Africa with 42% alcohol content. Ethanol is the almost alcohol found in *Sodabi*, with a proportion of 99%v/v.[19] After preparation, VIROSCOPE® has a final alcohol content of 30%.

The dosage of VIROSCOPE® for an adult human over 18 years of age is 40 ml twice a day (morning and evening), whereas the dosage for a human aged between 7 and 18 years is 20 ml twice a day (morning and evening), and the dosage for a child aged between 3 and 8 years is 05 ml twice a day (morning and evening). It is prohibited for children under three years of age and is authorised for pregnant and breastfeeding women. Previous studies had detected the presence of phytochemical groups present in VIROSCOPE® (polyphenols, hydrolyzable tannins, flavonoids, alkaloids and saponosides) and characterised rutin (195.37 ± 6.01 mg/g of dry extract) and gallic acid (121.31 ± 2.49 mg/g of dry extract). Additionally, other previous studies on the trial drug have characterised minerals, including calcium (8216 ± 1 mg/kg), magnesium (10389 ± 1 mg/kg), iron (83 ± 1 mg/kg), and zinc (19 ±1 mg/kg). Other *in vitro* studies have reported the anti-inflammatory and immune-cell proliferative properties of VIROSCOPE®.

METHODOLOGY

Ethical approval

Ethical approval for this study was provided by the Institutional Ethics Committee (IEC) of the Toxicology and Hydrology Laboratory of Cheikh Anta Diop University in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, 1985).

Two types of tests were performed on laboratory animals; they focused on the ready-to-use VIROSCOPE® phytomedicine, on the extract obtained after evaporation, and on the solvents recovered during evaporation.

Preparation of the product to be tested.
VIROSCOPE® batches tested.

Two bottles from different batches of VIROSCOPE® were tested. These two batches were prepared using 42% *Sodabi* (solvent 1 and solvent 2) purchased from two different suppliers in Togo.

Preparation of test substances

Each batch of ready-to-use VIROSCOPE® (300 ml per batch) was evaporated to dryness using a rotary evaporator (Heidolph Rotary Evaporator Hei-VAP, ProfiLab24 GmbH, Berlin, Germany) under vacuum at 65°C until a thick dry extract was obtained. The thick dry extract obtained using the rotary evaporator was subsequently dried in a laboratory incubator (POL-EKO Laboratory incubator CLW, ProfiLab24 GmbH, Berlin, Germany) at 40°C for 12h to obtain the final dry extract. This process enabled the separation of the dry extract and solvents which have been used to manufacture the present trial drug.

The dry extract obtained was crushed and the collected powder was dissolved in drinking water for various toxicity tests. Solvents 1 and 2 were obtained from the two batches of VIROSCOPE® and tested separately.

Experimental protocol
Lab animals

The animals consisted of albino Wistar rats, male and female, aged between 12 and 16 weeks with an average weight of 210 g; none of the animals showed any apparent signs of illness or suffering. All female rats were nulliparous and non-pregnant. Rats had unlimited access to food and water. The rat diet consisted of normal rat chow. The water came from the tap and underwent double filtration before being distributed into breeding bottles.

Acute toxicity

Acute toxicity assessment was performed in accordance with "OECD guideline 423 for the assessment of the acute toxicity of chemicals"[20]. This study focused on the ready-to-use VIROSCOPE®, and on the concentrated extracts obtained after evaporation. This guideline enables the estimation of the median lethal dose 50 (LD50),

which is the parameter used to evaluate the acute toxicity of a product and corresponds to the dose that kills half of the animals used in the experiment. This permitted the determination of toxicity during exposure to high doses, such as overdoses. Six female albino Wistar rats were divided into two groups (three rats per group). The rats in the first group received a dose of 5000 mg/kg body weight of the dry extract obtained after the concentration of VIROSCOPE® and dissolved in drinking water. Acute toxicity assessment was performed in accordance with "OECD guideline 423 for the assessment of the acute toxicity of chemicals." According to OECD guideline 423, page 4, paragraph 21, a dose level of 5000 mg/kg body weight may be considered, particularly when there is a strong likelihood that the results of such a test have direct relevance for protecting human or animal health or the environment. The rats in the second group received 2 mL / 100 g body weight of the ready-to-use VIROSCOPE® in liquid form. Before administering the products, the animals were fasted for 12 hours and weighed. The treated animals were observed individually during the first 30 min following the administration of the product and regularly for 24 h, with particular attention during the first 4 h. Observations were performed daily for 14 days to identify possible deaths and/or signs of toxicity.

During the test period, the animals were provided water ad libitum and normal rat chow. The food consumed was weighed and the volume of water consumed was measured daily. Two times weight of the rats were measured (on Day 7 and 14).

Subacute toxicity

This study highlights the biological and histopathological alterations that occur following repeated administration of VIROSCOPE®. Two bottles of VIROSCOPE® from two different batches were tested for the subacute toxicity. The evaluation focused on the ready-to-use VIROSCOPE®, the extract obtained after solvent evaporation, and the solvents recovered from the two batches of VIROSCOPE® after evaporation. The "OECD guideline 407 for the study of subacute toxicity of chemicals" [21] had been adopted. In accordance with this guideline, the evaluation focused on laboratory animals, including rats, and lasted 28 days. Thirty-nine (39) rats including 18 males and 21 females, were divided into six groups for males and 7 for females. The rats were randomly distributed into cages, each containing either three male rats or three female rats. The distributions of tested rats are presented in Table 1.

Administration

The products were orally administered seven days a week for 28 days using a feeding tube.

Table 1. Group of rats used for the subacute toxicity test

Group of rats	Male rats	Female rats
Group 1 (control)	drinking water	drinking water
Group 2	ready-to-use VIROSCOPE®	ready-to-use VIROSCOPE®
Group 3	solvent 1 (series N°1)	solvent 1 (series N°1)
Group 4	dry extract dissolved in drinking water at a dose of 100 mg/kg	solvent 2 (series N°2)
Group 5	dry extract dissolved in drinking water at a dose of 500 mg/kg	dry extract dissolved in drinking water at a dose of 100 mg/kg
Group 6	dry extract dissolved in drinking water at a dose of 1000 mg/kg	dry extract dissolved in drinking water at a dose of 500 mg/kg
Group 7	-	dry extract dissolved in drinking water at a dose of 1000 mg/kg

Rat tracking

The treated rats were observed individually during the first 30 min following the administration of the product and regularly during the day to record the number of deaths and clinical signs of toxicity. Various symptoms of intoxication were recorded daily. The body weight of each rat was determined at the end of each week and 24-hour urine was collected to measure the protein and glucose levels. At the end of the test period, the rats were anaesthetised (xylazine 2% + ketamine 100 mg/mL), and blood was collected in EDTA tubes, tubes with sodium fluoride, and dry tubes for haematological and biochemical analyses. The rats

were then sacrificed, an autopsy was performed, and the organs (kidney and liver) were removed and preserved in 10% buffered formalin for histopathological analysis.

Statistical analysis

Statistical analysis was performed using R software version 3.6.1. The difference in mean for number of rats (n = 3) was tested using Student's t-test, with a confidence of 95%.

RESULTS

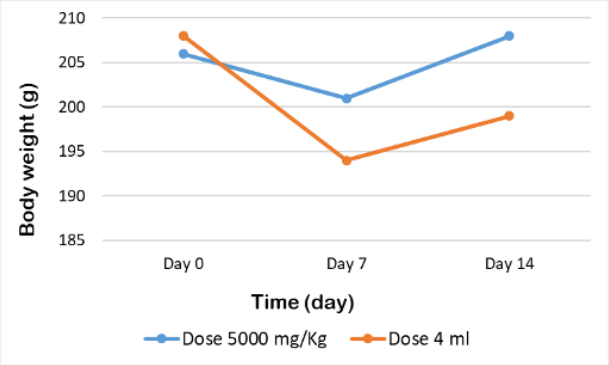
Acute toxicity

At the end of the experiment, no death was recorded for the two batches of VIROSCOPE®, and no signs of toxicity were observed in the rats that received a dose of 5000 mg/kg. The changes in body mass, food intake, and water consumption were normal (Figure 1), (Table 2). For rats that received trial drug at a dose of 2 ml/100 g body weight, a state of lethargy was observed, accompanied by drunkenness, which disappeared after 4 h. The estimated LD50 for this study was greater than 5000 mg/kg because, at this dose, there were no deaths or other signs of toxicity that could be attributed to the trial drug.

Subacute toxicity

The main symptom recorded was drunkenness.

Figure 1. Evolution of body weight of rats treated for acute toxicity (n=3)



In all rats that received the ready-to-use VIROSCOPE® and the solvent, a state of lethargy accompanied by drunkenness was observed, and this lethargy and drunkenness disappeared after 2 h. However, in males, we observed a significant reduction in food consumption in groups of rats that received the solvent, VIROSCOPE® and a dose of 1000 mg/kg of the dry extract (Table 3). Among the female rats from group 2 who received solvent 2, two (2) out of three (3) died before the end of the study. [Solvents 1 and 2 are both traditionally prepared 42% ethanol, purchased from different manufacturers. Based on the result of this study, VIROSCOPE® is now prepared using traditional ethanol purchased from the producer of solvent 1] Moreover, there was no significant difference in water consumption between control and treated rats (Tables 5 and 6). No other signs of toxicity were recorded or observed during the 28 days of observation. Therefore, the effect of trial drug, at repeated doses over a long period of 28 days, would also be without a major risk.

VIROSCOPE®; solvents 1 and 2; 100, 500, and 1000 mg/kg body weight of the dry extract dissolved in drinking water did not induce haematological disorders affecting white blood cells, red blood cells, haemoglobin, hematocrits, mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin, and platelets (Table 7).

Biochemical parameters are usually measured to assess the possible toxic effects of herbal drugs on the liver and kidney function. Urea and creatinine are first-line screening tests for kidney functions while AST and ALT are first-line screening tests for liver functions. Compared to the control rats, the ready-to-use VIROSCOPE®, solvents 1 and 2, 100, 500, and 1000 mg/kg body weight of the dry extract dissolved in drinking water did not induce any significant differences in biochemical parameters such as blood sugar, AST, ALT, uraemia, and creatinine.

Table 2. Average water and food consumption of rats treated for acute toxicity (n=3)

Parameter	Dose	Day 0	Day 7	Day 14
Food (g)	5000 mg/kg	10.23	18.79 ± 4.89	30.70 ± 12.04
	2 mL/100g	15.68	12.64 ± 3.22	23.49 ± 11.74
Water (ml)	5000 mg/kg	30	25.4 ± 5.26	31.43 ± 12.60
	2 mL/100g	33.33	27.38 ± 6.37	30.48 ± 14.84

Table 3. Variations in food consumption of male rats subjected to subacute toxicity testing

Group of rats	Food consumption per week (g)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control rats	31.42	33.05 ± 3.99	31.44 ± 5.62	30.21 ± 5.33	37.78 ± 7.26
Solvent 1	35.07	32.25 ± 6.79	34.14 ± 5.71	30.86 ± 6.83	30.69 ± 4.42*
Ready-to-use VIROSCOPE®	32.29	26.90 ± 4.44*	25.40 ± 7.65	27.13 ± 3.65	25.45 ± 4.12**
Dose of 100 mg/kg	29.46	29.31 ± 5.23	28.62 ± 4.35	30.16 ± 5.98	32.99 ± 5.13
Dose of 500 mg/kg	31.29	29.80 ± 3.37	29.33 ± 8.34	28.40 ± 7.66	31.65 ± 5.72
Dose of 1000 mg/kg	30.13	24.15 ± 7.78*	16.09 ± 5.80*	17.71 ± 7.81*	16.56 ± 2.70***

*, p-value < 0.005 considered significant with n = 3 (Student's t-test), i.e. a significant reduction in food consumption in male rats compared to control rats

Table 4. Variations in food consumption of female rats subjected to subacute toxicity testing

Group of rats	Food consumption per week (g)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control rats	33.04	35.67 ± 4.64	30.38 ± 4.18	32.37 ± 2.99	30.94 ± 2.94
Solvent 2	29.53	17.67 ± 7.59***	5.62 ± 4.57***	5.76 ± 3.02**	7.49 ± 2.96***
Ready-to-use VIROSCOPE®	31.77	29.17 ± 5.62	24.82 ± 7.41	23.55 ± 6.58**	23.41 ± 2.76*
Dose of 100 mg/kg	25.74	31.51 ± 4.51	27.96 ± 4.63	27.86 ± 4.27	27.78 ± 3.29
Dose of 500 mg/kg	33.33	30.65 ± 7.82	35.08 ± 2.79	27.37 ± 3.26	26.25 ± 5.94
Dose of 1000 mg/kg	25.12	20.64 ± 3.54***	17.12 ± 2.42**	18.09 ± 3.27***	23.49 ± 2.73**

*, p-value <0.005 considered significant with n = 3 (Student's t-test), i.e. a significant reduction in food consumption in female rats compared to control rats

Table 5. Water consumption of male rats subjected to subacute toxicity testing

Group of rats	Weekly water consumption (ml), n=3				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control rats	36.67	35.95 ± 6.93	33.57 ± 15.35	44.52 ± 10.57	46.90 ± 7.72
Solvent	36.67	32.86 ± 3.00	35.00 ± 5.44	40.48 ± 3.15	45.71 ± 7.63
Ready-to-use VIROSCOPE®	33.33	29.52 ± 3.00	29.05 ± 4.70	39.05 ± 4.99	35.29 ± 8.33
Dose of 100 mg/kg	38.33	36.19 ± 2.84	35.95 ± 6.79	40.95 ± 5.35	38.38 ± 5.43
Dose of 500 mg/kg	41.67	36.67 ± 5.53	35.95 ± 6.07	39.05 ± 10.53	50.71 ± 9.37
Dose of 1000 mg/kg	35.00	36.67 ± 7.01	38.31 ± 15.29	39.29 ± 9.76	45.00 ± 8.16

Table 6. Water consumption of female rats subjected to subacute toxicity testing

Group of rats	Weekly water consumption (ml), n=3				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control rats	25.00	30.24 ± 3.39	21.19 ± 11.81	33.57 ± 4.13	29.76 ± 5.65
Solvent	26.67	24.76 ± 4.24	13.10 ± 6.27	13.00 ± 3.45	15.95 ± 2.70
Ready-to-use VIROSCOPE®	33.33	34.57 ± 5.61	32.38 ± 7.13	37.38 ± 3.31	41.19 ± 5.33
Dose of 100 mg/kg	33.33	35.48 ± 8.75	28.81 ± 3.15	34.76 ± 3.90	35.95 ± 9.22
Dose of 500 mg/kg	26.67	25.71 ± 5.68	28.33 ± 4.81	23.10 ± 7.23	26.67 ± 8.44
Dose of 1000 mg/kg	30.00	33.81 ± 9.32	30.14 ± 11.62	33.81 ± 6.58	39.52 ± 5.07

Figure 2. Evolution of body weight of male rats subjected to the subacute toxicity testing over 28 days. Each point corresponds to the weekly average body weight of three rats

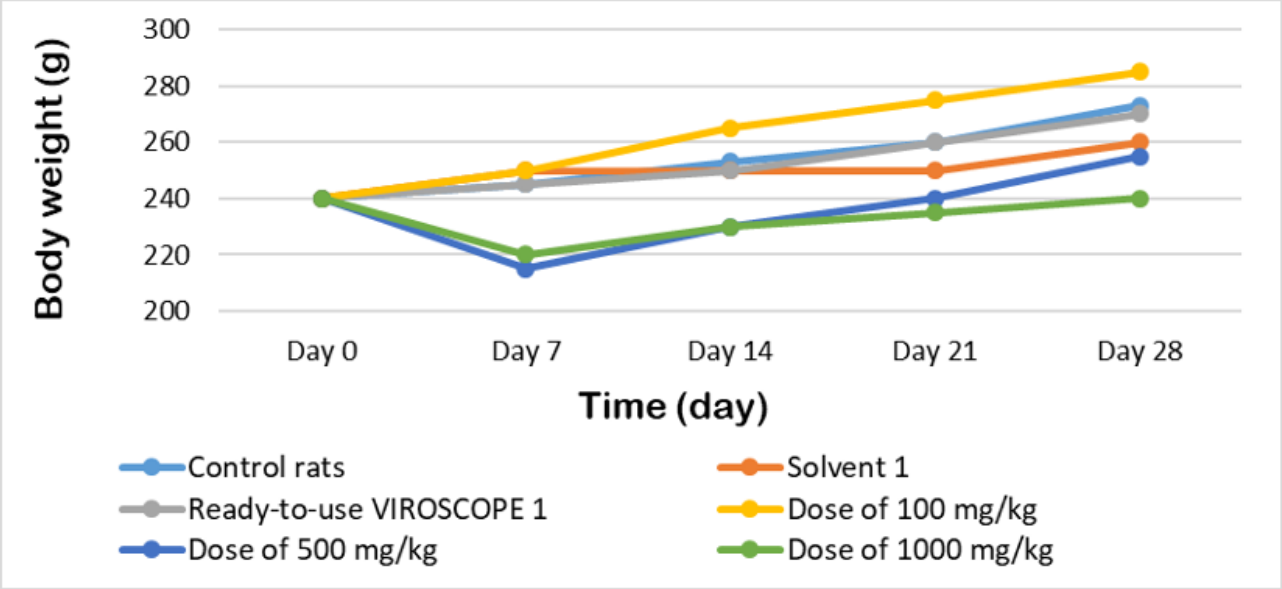


Figure 3: Evolution of body weight of female rats subjected to subacute toxicity testing over 28 days. Each point corresponds to the weekly average body weight of three rats

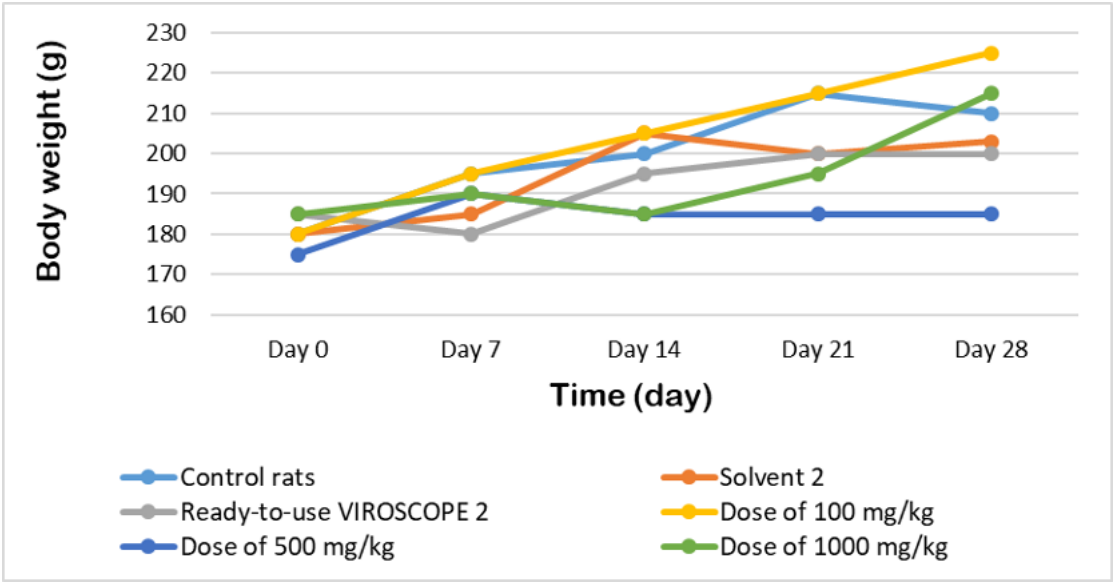


Table 7. Haematological parameters

Group of rats		WBC	RBC	HGB	HCT	MCV	MCHC	MCH	PLT
Control rats	**Male**	5.85 ± 1.78	7.69 ± 0.64	13.57 ± 0.97	50.55 ± 5.16	68.62 ± 1.20	17.98 ± 0.21	27.14 ± 0.14	1011.33 ± 265.17
	Female	7.12 ± 1.82	7.36 ± 0.39	13.4 ± 0.20	48.63 ± 1.17	66.2 ± 2.39	18.27 ± 0.68	27.60 ± 0.52	995.67 ± 129.86
solvent	**Male**	6.03 ± 0.06	7.93 ± 0.46	13.75 ± 0.64	50.00 ± 2.40	63.15 ± 0.64	17.35 ± 0.21	27.50 ± 0.00	897.50 ± 38.89
	Female	5.70 ± 0.48	6.82 ± 0.45	12.6 ± 0.78	45.20 ± 3.30	66.2 ± 1.31	18.47 ± 0.31	27.90 ± 0.26	924.00 ± 70.38
Ready-to-use VIROSCOPE®	**Male**	6.12 ± 1.68	8.10 ± 0.45	14.33 ± 0.49	52.53 ± 1.95	64.87 ± 1.42	17.70 ± 0.72	27.30 ± 0.60	1050.67 ± 109.61
	Female	5.96 ± 2.59	6.77 ± 0.80	12.20 ± 1.35	44.67 ± 4.99	66.03 ± 0.55	18.50 ± 0.26	28.00 ± 0.56	946.67 ± 164.04
Dose of 100 mg/kg	**Male**	4.94 ± 2.36	7.79 ± 0.26	14.13 ± 0.45	50.30 ± 1.66	64.57 ± 1.33	18.16 ± 0.12	28.06 ± 0.40	874.00 ± 112.58
	Female	6.14 ± 1.95	6.67 ± 0.60	12.33 ± 0.96	43.27 ± 3.56	65.17 ± 4.28	18.60 ± 1.04	28.53 ± 0.31	899.33 ± 142.03
Dose of 500 mg/kg	**Male**	9.50 ± 2.02	7.68 ± 0.56	13.43 ± 0.55	47.83 ± 2.55	62.37 ± 1.40	17.53 ± 0.70	28.10 ± 0.46	953.67 ± 55.32
	Female	5.74 ± 0.59	6.16 ± 0.45	11.7 ± 1.41	40.60 ± 4.10	65.85 ± 1.77	18.95 ± 0.92	28.00 ± 0.57	887.00 ± 101.82
Dose of 1000 mg/kg	**Male**	10.98 ± 3.81	8.28 ± 0.82	14.75 ± 1.20	52.75 ± 6.29	63.65 ± 1.34	17.85 ± 0.35	28.05 ± 1.06	897.50 ± 318.91
	female	9.68 ± 3.87	6.95 ± 0.25	12.97 ± 0.64	45.07 ± 2.73	64.77 ± 1.90	18.63 ± 0.23	28.76 ± 0.55	944.67 ± 24.70

WBC, White blood cell; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin; PLT, platelet

Table 8. Biochemical parameters

Group of rats		Blood sugar (g/L)	AST (UI/L)	ALT (UI/L)	Uremia (g/L)	Creatininemia (mg/L)
Control rats	**Male**	1.74 ± 0.81	191 ± 111.00	128.00 ± 68.39	0.34 ± 0.21	5.74 ± 0.71
	Female	2.24 ±0.53	207.67 ± 22.14	92.00 ± 12.49	0.51 ± 0.12	5.49 ± 0.12
solvent	**Male**	1.97 ± 0.35	204.00 ± 33.94	112.00 ± 9.90	0.47 ± 0.08	5.64 ± 0.01
	Female	2.13 ± 0.43	178.67 ± 11.93	89.00 ± 8.89	0.44 ± 0.09	5.51 ± 0.13
Ready-to-use VIROSCOPE®	**Male**	1.97 ± 0.57	179.33 ± 13.61	103.67 ± 12.42	0.44 ± 0.07	4.80 ± 0.00
	Female	2.12 ± 0.41	180.67 ± 20.40	107.33 ± 17.67	0.35 ± 0.12	5.46 ± 0.29
Dose of 100 mg/kg	**Male**	2.19 ± 0.82	171.00 ± 27.50	136.67 ± 18.61	0.52 ± 0.12	5.24 ± 0.99
	Female	2.03 ± 0.37	142.00 ± 14.00	104.33 ± 4.73	0.51 ± 0.02	5.46 ± 0.47
Dose of 500 mg/kg	**Male**	2.19 ± 0.43	160.00 ± 15.72	163.00 ± 60.61	0.39 ± 0.03	4.92 ± 0.17
	Female	1.98 ± 0.00	148 ± 8.49	97.5 ± 14.85	0.59 ± 0.16	5.49 ± 0.00
Dose of 1000 mg/kg	**Male**	3.23 ± 0.40	152.5 ± 24.75	144.5 ± 36.06	0.65 ± 0.23	5.62 ± 0.28
	female	2.46 ± 0.53	144.67 ± 12.90	118.33 ± 3.06	0.45 ± 0.10	5.91 ± 0.43

ALT, transaminases alanine transaminase; AST, aspartate transaminase

Therefore, VIROSCOPE® would have no harmful effects on kidney and liver function (Table 8).

Histopathological analysis of liver cells showed dilation of the centri-lobular veins and sinusoids in all rats, except for those that received VIROSCOPE®, in which the presence of Councilman bodies, which may be of toxic origin was reported. In addition, arteriolar congestion of renal cells was observed in all rats, including control rats.

DISCUSSION

Acute toxicity

According to the Globally Harmonised System, VIROSCOPE® belongs to Category 5 of the United Nations classification,[22] that is, practically zero acute toxicity, according to Hodge and Sterner.[23] The ready-to-use VIROSCOPE®, would be non-toxic for a single dose in rats up to a dose of 2 ml/100 g of body weight (20 ml/kg), well above the human dosage which is 1.14 ml/kg per day. Like us, several studies have also reported medicinal plants and phyto medicines with LD > 5000 mg/Kg in Nigeria, Saudi Arabia, India, and China.[24–27]

Subacute toxicity

The reduction in the quantity of food consumed by the rats that received VIROSCOPE® (Tables 3 and 4) could be partly attributed to the 42% *Sodabi* (composed of 99% ethanol), used to prepare VIROSCOPE®. Indeed, ethanol has been shown to induce anorexia in humans and rats.[28–30]

Among the female rats from group 2 who received solvent 2, two (2) out of three (3) died before the end of the study. Therefore, solvent 2 would be slightly toxic and must be replaced with solvent 1 during further preparation of the trial drug. For explanation, solvents 1 and 2 are both 42% *Sodabi*, (a traditionally distilled palm wine containing 99% ethanol), purchased from different manufacturers in Togo.

Similarly, studies in India, Colombia, the Republic of Korea, and Pakistan have reported medicinal plant preparations having no negative impact on haematological and biochemical parameters.[24,31–33]

Gender differences are an important issue in drug research and development. Studies have shown that differences observed between genders in terms of medications can be explained by variations in plasma protein levels, plasma volume, oestrogen and various enzyme levels, drug transporter function, excretion activity and other different factors between males and females.[34–36] These reasons could explain the slight differences between the results obtained in male and female rats for control and treated rats.

Additional studies would enable elucidation of the role of VIROSCOPE® in the formation of councilman bodies. The histopathological results suggest that the dilation of the centri-lobular veins and sinusoids, as well as the arteriolar congestion observed, cannot be attributed to VIROSCOPE® because the same results were found in both the rats that received the trial drug and in the controls that had received only drinking water. These results could be explained by the storage conditions of the biopsy specimens (liver and kidneys).

CONCLUSION

Based on the acute toxicity results, the components of VIROSCOPE® can be considered harmless in rats, even at a high dose of approximately 5000 mg/kg. It can also be considered safe in the short term at the dosage indicated on the bottle which is 1.14 ml/kg a day. In fact, there were no signs of toxicity at a dose of 20 ml/kg. The effect of VIROSCOPE®, at repeated doses over a long period of 28 days, would also be without major risk. According to histopathological results, the trial drug would not be significantly harmful to liver and kidney cells. However, the drug must be administered strictly with respect to the indicated dosage of 40 ml twice a day (morning and evening) for adult patients, and the quality of the solvent (*Sodabi*) used to prepare it must be strictly controlled. Finally, a very long treatment period exceeding 2 months is not recommended.

Perspectives

The authors plan to begin clinical trials on the therapeutic use of VIROSCOPE®.

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