Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Pharmacology and Toxicology

ISSN 1816-496X
DOI: 10.3923/jpt.2024.1.12

Research Article
Acute and Sub-Acute Toxicity of Kabran01, an Ivorian Antidiabetic Herbal Preparation in Wistar Rats

[1]Kanga Sita N'Zoue, [1]Camille Koffi, [1]N'goran Mathieu Kouame, [2]Brahima Doukoure and [1]Mamadou Kamagate

[1]Laboratory of Clinical Pharmacology, UFR Medical Sciences Bouaké, Alassane Ouattara University, Bouaké, Côte d'Ivoire
[2]Laboratory of Pathological Anatomy, UFR Medical Sciences Abidjan, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire

Abstract

Background and Objective: Kabran01 is a traditional remedy composed of twelve plants used by Ivorians for the treatment of diabetes 20 years ago. The present study assessed the safety of aqueous and hydroethanolic Kabran01-extracts by evaluating its potential toxicity after acute and subacute administration in rats. **Materials and Methods:** For the acute study, the lyophilised aqueous (decocted, infused) and hydroethanolic extracts of Kabran01 was administered to rats (3 months old, 150-250 g) in single doses of 2000 or 5000 mg/kg by gavage. General behaviour, signs of pain and mortality were determined up to 14 days. Certain biochemical and haematological parameters were determined at the end of 14 days. After the 28 days of daily administration of the Kabran01-extracts (1000 mg/kg), selected biochemical and haematological parameters and microscopy of vital organs (heart, liver, pancreas and kidney) of treated-rats were determined. **Results:** Oral administration of Karban01 at a single dose of 2000 mg/kg or 5000 mg/kg and repeated dose of 1000 mg/kg for 28 days did not alter biochemical and haematological parameters and blood ionogram compared with control rats. No adverse effects on general behaviour and no mortality were observed in both acute and subacute studies. Histological examinations showed that these higher doses of Kabran01-extracts induce minimal ectasia, inflammation, oedema of the heart and minor hypertrophy of cardiomyocytes. The Kabran01 caused moderate hepatic ectasia, pyknosis and steatosis, as well as minimal inflammation and ectasia of the pancreas and minimal to moderate renal tubular dilatation and necrosis. **Conclusion:** The use of Kabran01 at therapeutic doses in diabetes must therefore be monitored in order to better assess its risk/benefit ratio in real-life conditions.

Key words: Kabran01, traditional remedy, diabetes, toxicity, vital organs

Citation: N'Zoue, K.S., C. Koffi, N.M. Kouame, B. Doukoure and M. Kamagate, 2024. Acute and sub-acute toxicity of Kabran01, an Ivorian antidiabetic herbal preparation in Wistar rats. J. Pharmacol. Toxicol., 19: 1-12.

Corresponding Author: Camille Koffi, Laboratory of Clinical Pharmacology, UFR Medical Sciences Bouaké, Alassane Ouattara University, Bouaké, Côte d'Ivoire Tel: (+225)0707194024

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Traditional medicine is a set of practices, methods, knowledge and beliefs used to diagnose, prevent and treat disease[1]. This historical practice is transmitted orally from generation to generation[2]. Today, complementary and alternative medicine is experiencing resurgence around the world[3]. In Africa, especially in Côte d'Ivoire, several traditional healers offer recipes that are supposed to be effective against various pathologies[4]. However, these natural products had generally not been scientifically assessed never to determine their toxicity, their tolerance nor their pharmacological activities[5]. Thus, their benefit/risk balance is a need. Herbal medicine research has become one of the greatest scientific concerns to overcome the lack of scientific evidence of therapeutic efficacy and the toxicity level of natural substances use[6-8]. These researches for scientific evidence would lead to the proposal of effective and well tolerated therapies in specific indications. For example, Faca® is natural product made in Burkina Faso and sold in pharmacies in Sub-Saharan Africa[9].

In practice, using medicinal plants offered by traditional healers could expose people to intoxication risk. Indeed, surveys of traditional healers have revealed that the herbal use could cause intoxications and complications of pathologies. Drug poisonings in humans are believed to be caused by botanical identification errors or mislabeling of plant material, contamination of medicinal plants with microorganisms, fungal toxins, pesticides and heavy metals and inappropriate treatments made by traditional practitioners[10]. The kidneys are particularly susceptible to damage from toxins, including toxic components of medicinal plants[11].

The Kabran01 is a traditional remedy containing twelve plants including *Paullinia pinnata*, *Persea americana*, *Cassia siamea* and *Cymbopogon citratus*. This herbal remedy is used by Ivoirians for the treatment of diabetes 20 years ago. For the treatment of diabetes, a handful of approximately 14 g of powder from this natural recipe is infused in 1 L of hot water and administered orally throughout the day. The daily dose of Kabran01 is therefore around 200 mg/kg for a person weighing 70 kg. Preliminary studies seem to show short-term efficacy and tolerance in rats. However, we do not have any medium and long-term data in this chronic disease. In order to rule out its safety profile during prolonged use, this study was conducted to assess the acute and subacute toxicity of aqueous and hydroethanolic Kabran01-extracts in rats.

MATERIALS AND METHODS

Study area: This study was carried out from February 2022 to September 2023, i.e., for one year and seven months at the Laboratory of Clinical Pharmacology, UFR Medical Sciences Bouaké, Alassane Ouattara University, Bouaké, Ivory Coast.

Chemicals: Chemicals used were ethanol (Quimicen®, Spain), lidocaïne gel (AstraZeneca®, UK), Tween® 80 (Sigma-Aldrich, Germany) and biochemicals kits (Biorad, France). Paraffin oil, carbon tetrachloride (Spectrosol® BHD chemicals Ltd., pool, England) and solvents were of highest grade commercially available.

Animals: Male and female Wistar rats (3-month-old, 150-250 g) were procured from animal resources of Medical Sciences Department, Alassane Ouattara University, Bouaké, Côte d'Ivoire. The rats were provided with standard pellet diet and water *ad libitum*. They were kept in cages under standard environmental conditions of 12 hrs light/dark cycles and room temperature of 25±2°C during all experiments.

Ethical consideration: The present study was approved by the Scientific Committee of the UFR Medical Sciences Bouaké, Alassane Ouattara University, Bouaké, Ivory Coast (N°:2021/MESRS/UAO/UFRSMOK) and all experimental protocols were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals[12].

Preparation of the herbal formulation (Kabran01): The traditional recipe Kabran01 is an association of twelve Ivorian medicinal dishes which secret preparation and blending is held by the traditional healers. The fresh leaves of these plants are crushed in water then dried at room temperature away from sunlight. For this study, 50 g powder of the traditional recipe were used for decoction, infusion or hydroalcoholic extraction (70/30, ethanol/water). The decocted (DEK) and infused (IEK) extracts were lyophilized (Chris Martin 4200, France). The hydroalcoholic extract (HEK) was evaporated at 40°C in a circulating air oven (Memmert®, Germany). All dried and freeze-dried extracts were stored at 4°C until use.

Experimental design: The study was conducted in strict accordance with the guidelines of Organization for Economic Cooperation and Development for testing of chemicals, acute toxic class method (OECD 423) and sub-chronic class method (OECD 407).

Acute toxicity study: Wistar rats were divided into four groups (I-IV) of three each (n = 3/group) and fasted overnight. Thus, animals in each group were treated 1-1 at 48 hrs intervals. Orally, group I received vehicle (2% Tween 80, v/v), groups II, III and IV received a single dose of 2000 mg/kg and 5000 mg/kg b.wt., of decocted (DEK), infused (IEK) and hydroalcoholic (HEK) extracts of the Kabran01. After administration, the rats were continuously observed every 1 hr for 4 days. The food was suspended for a period of 3-4 hrs. Additional observation was made every 24 hrs for 15 days to identify any changes such as weight, diet, tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma, decreased respiratory rate and lethality. Also, the hair, skin, eyes and mucous membranes of the rats were observed.

Sub-acute toxicity study: Wistar rats were randomly divided into four groups (I-IV) of six animals (three females and three males). Orally, group I, received the vehicle (2% Tween 80, v/v), groups II, III and IV, respectively received repeated daily doses of 1000 mg/kg b.wt., body weight of decocted, infused and hydroalcoholic extracts of mixture of Kabran01 for 28 consecutive days.

Blood and tissue collection: At the end of the experiments, rats fasted overnight (control and experimental rats) were sacrificed under light ether anaesthesia by cervical decapitation. Blood samples were taken by cardiac puncture before incision of the abdomen, 5 mL of blood samples were collected in two types of tubes, serum red tubes without coagulation activator and purple tubes with anticoagulant (EDTA). After blood sampling, their vital organs (liver, heart, kidney and pancreas) were carefully dissected, measured and weighed.

Biochemical and haematological parameters: Biochemical parameters were analyzed on serum obtained from whole blood samples collected in red tubes and fractionated by centrifugation at 3000 rpm for 15 min. Serum was aliquot into 1.5 mL microvials and serum glucose, triglycerides (TG), total cholesterol, urea, creatinine, aspartate aminotransferase (AST), Alanine Aminotransferase (ALT) and alkaline phosphatase (AP) were enzymatically determined by standard methods using specific kits suitable for a URIT8260® Chemistry System (Diamond Diagnostics Inc., USA) using automated procedures. Sodium (Na^+), potassium (K^+) and chlorine (Cl) were analyzed using electrolyte analyzer ISE 3000® (SFRI Medical diagnostics, France) with compatible ISE reagent packs.

Haematological parameters were determined on whole blood samples collected in violet tubes using an XN-550® blood auto-analyzer (SYMEX, France), incorporating flow fluoro-cytometry, hydrodynamic focusing and the cyanide-free SLS method for haemoglobin measurement.

Histopathological examinations: The collected tissues (liver, heart, kidneys and pancreas) were fixed in a buffered solution of formaldehyde (10%), dehydrated by a solution of ethanol in series, diaphonized with ethanol-benzene and surrounded by paraffin. Micrometric sections were stained with Haematoxylin and Eosin, examined under a light microscope at low and high magnification and photographed. Lesions are arbitrarily classified as minimal, moderate or severe according to their severity.

Statistical analysis: The results were expressed as Mean±Standard Error of Mean (SEM). Statistical analysis was performed using GraphPad Prism 5.0® software. The difference between groups was assessed by Analysis of Variance (ANOVA), followed by the Turkey's test or Bonferroni post-tests. Differences were considered statistically significant for $p<0.05$.

RESULTS

Acute toxicity of Kabran01 at 2000 mg/kg, PO: After administration of Kabran01, the weights of the rats were comparable throughout the 14-day observation period (Fig. 1a). Administration of decocted and infused extracts of Kabran01 induced high water consumption in rats (Fig. 1b). Also, food consumption was comparable in all groups of rats during the 14 days of observation (Fig. 1c).

The administration of Kabran01 (2000 mg/kg, b.wt.) does not cause any mortality, convulsion, lethargy, diarrhoea, drowsiness, coma and other abnormal behaviours or movements in rats. It did not induce modifications in rat's body weight, water and food consumption. Also, the hair, skin, eyes and mucous membranes of the rats were not modified by Kabran01.

The biochemical parameters, blood urea and creatinine were statistically comparable between the groups. So were transaminases (ALT, AST and AP), total cholesterol and triglycerides were similar. The blood ionogram of the decocted, infused and hydroalcoholic Kabran01 were statistically not different at day-14 from that of the control. Finally, hemogram and hematimetic parameters were not different in the 3 groups compared to the control data (Table 1). Table 2 showed that administration of infused

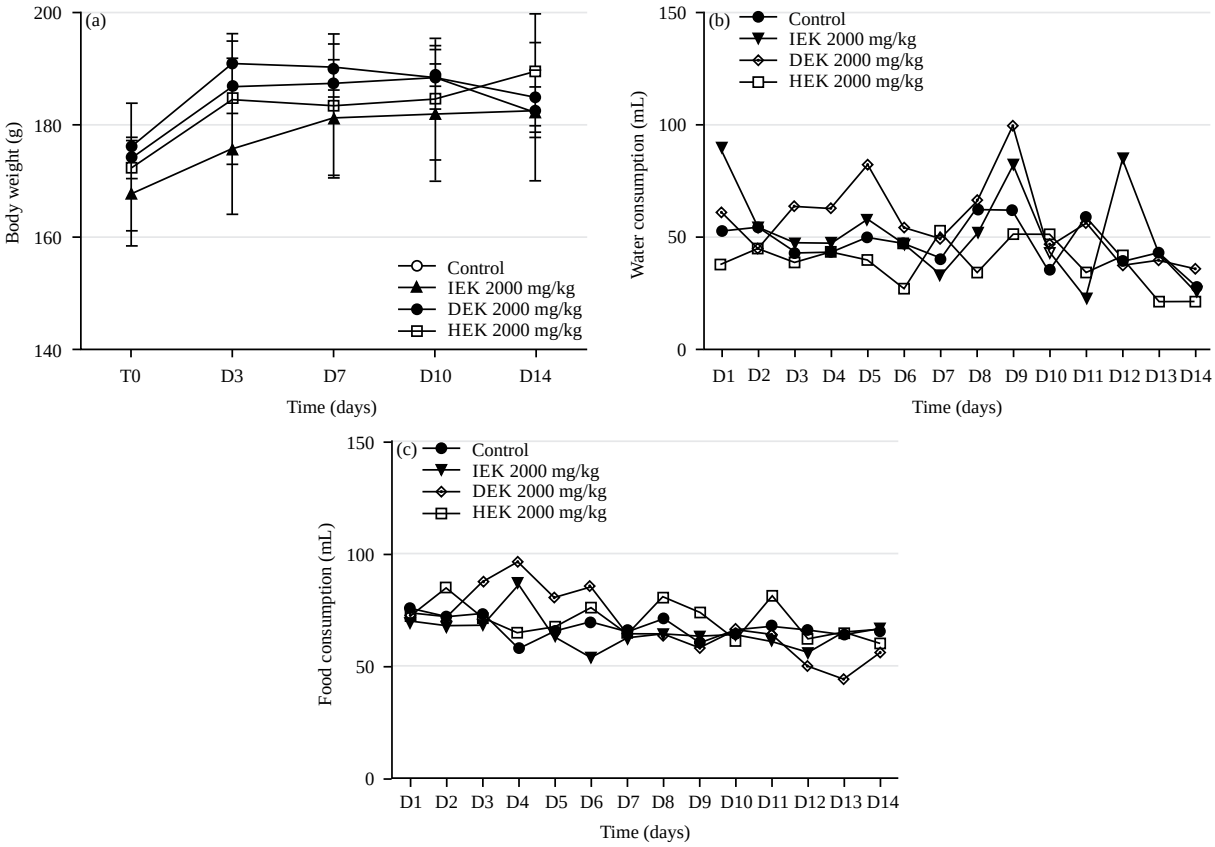

Fig. 1(a-c): Acute effect of Kabran01 (2000 mg/kg, b.wt.) on clinical parameters of rats (n = 3/group), (a) Body weight of rats, (b) Water consumption and (c) Food consumption

Results are express as Mean±SEM, Statistic analysis: ANOVA followed by Bonferroni's post tests, $p<0.05$, Kabran01 has no significant influence on body weight, water and food consumption of rats

Table 1: Acute effect of Kabran01 at 2000 mg/kg on blood parameters of rats at day-14

	Experimental groups of rats (n = 3/group)			
Parameter	Control	DEK 2000 mg/kg	IEK 2000 mg/kg	HEK 2000 mg/kg
Biochemical				
CPK (UI/L)	142.00±6.66	207.33±53.51	98.67±0.88	131.67±14.62
Urea (mmol/L)	0.42±0.01	0.42±0.03	0.34±0.08	0.45±0.02
Creatinine (µmol/L)	4.00±0.00	4.00±0.58	4.67±0.33	5.00±0.00
Cholesterol (UI/L)	0.58±0.26	0.78±0.03	0.86±0.12	0.69±0.03
Triglycerides (UI/L)	0.90±0.04	0.99±0.02	0.99±0.01	0.90±0.03
ALT (UI/L)	107.67±5.36	97.33±7.54	97.33±9.53	100.00±9.71
AST (UI/L)	525.67±33.67	417.33±86.37	441.00±83.81	327.00±85.98
PAL (UI/L)	195.67±10.17	79.67±35.71	140.67±64.89	116.67±17.17
Ionogram				
Natremia (mmol/L)	9.20±0.95	9.43±1.54	9.93±0.88	9.83±0.90
Kalemia (mmol/L)	9.20±0.95	9.43±1.54	9.93±0.88	9.83±0.90
Chloremia (mmol/L)	108.33±3.71	102.67±2.03	103.67±3.71	107.67±3.38
Haematological				
Erythrocytes (10^6/mm^3)	13.60±0.45	6.34±0.18	7.17±0.29	10.17±0.14
Leucocytes (10^3/mm^3)	13.60±0.52	5.66±0.14	7.27±0.29	7.24±0.18
Haemoglobin [Hg (g/dL)]	13.60±0.46	5.94±0.23	7.04±0.24	8.06±0.42
Platelets (10^3/mm^3)	806.33±42.14	724.00±188.64	1017.00±142.59	957.66±115.96

Results are expressed as Mean±EMS, Statistical analysis (ANOVA followed by Tukey's Multiple Comparison Test, $p<0.05$), DEK: Decocted extract of Kabran01, IEK: Infused extract of Kabran01, HEK: Hydroethanolic extract of Kabran01, CPK: Creatine phosphokinase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase and N: Number of rats in group. There was no difference between the values of the biochemical, ionographic and haematological parameters in the different experimental groups of rats

Table 2: Acute effect of Kabran01 at 2000 mg/kg on relative organ mass of rats at day-14

		Vital organs from experimental groups of rats (n = 3/group)			
	Rat (g)	Heart (g%)	Pancreatic (g%)	Liver (g%)	Kidneys (g%)
Control	182.27±5.85	0.79±0.02 (0.43)	5.61±0.37 (3.08)	1.04±0.01 (0.57)	0.77±0.08 (0.42)
DEK 2000 mg/kg	184.80±6.56	0.83±0.09 (0.45)	5.58±0.90 (3.01)	1.12±0.04 (0.61)	0.83±0.05 (0.45)
IEK 2000 mg/kg	182.40±15.83	1.51±0.06 (0.83)***	6.36±0.24 (3.57)	1.59±0.06 (0.88)***	1.25±0.07 (0.69)*
HEK 2000 mg/kg	189.40±13.63	1.52±0.10 (0.80)***	6.34±0.76 (3.32)	1.69±0.12 (0.89)***	1.38±0.11 (0.73)**

Results are expressed as Mean±EMS. Statistical analysis: ANOVA followed by Bonferroni's Multiple Comparison Test, *p<0.05,**p<0.01, ***p<0.001, DEK: Decocted extract of Kabran01, IEK: Infused extract of Kabran01, HEK: Hydroethanolic extract of Kabran01 and N: Number of rats in group

Table 3: Acute effect of Kabran01 at 2000 mg/kg on histology of vital organs of rats at day-14

		Experimental groups of rats (n = 3/group)			
Organs	Lesions	Control	DEK 2000 mg/kg	IEK 2000 mg/kg	HEK 2000 mg/kg
Heart	Hypertrophy (cardiomyocytes)	-			
	Ectasia	-	Minimal (33%)	Minimal (100%)	
	Oedema	-			
	Congestion	Minimal (33%)	Minimal (33%)	Minimal (67%)	Minimal (100%)
	Inflammation	-	-	-	
Liver	Necrosis	-		Pyknosis (67%)	Pyknosis (67%)
	Fibrosis	-			
	Steatosis	-	Minimal (67%)	Moderate (33%)	Minimal (67%)
	Congestion	Minimal (100%)	Minimal (67%)	Moderate (100%)	
	Ectasia	Moderate (100%)	Minimal (67%)		
	Peliosis	-			
	Inflammation	Minimal (100%)	Minimal (67%)		
Pancreas	Necrosis	-	-	-	-
	Atrophy	-	-	-	Moderate (33%)
	Cystic Fibrosis	-	-	-	-
	Ectasia	-	-	Moderate (33%)	Minimal (100%)
	Fibrosis	-	-	-	-
	Steatosis	-	-	-	-
	Hypertrophy	-	-	-	-
	Inflammation	-	-	-	-
Kidneys	Glomeruli dilatation	-	-		
	Tubules dilatation	-	-		Minimal (67%)
	Tubular necrosis	-	-	Moderate (67%)	
	Ectasia	Minimal (100%)	Minimal (33%)		Minimal (67%)
	Oedema	-	-		
	Congestion	Minimal (67%)	Minimal (100%)		
	Inflammation	Minimal (33%)	Moderate (67%)	Moderate (33%)	

Microscopic lesions observed in organs from rats treated with Kabran01 are presented as a proportion (number of rats with lesions observed/total number of rats treated), lesions are arbitrarily classified as minimal, moderate or severe according to their severity, DEK: Decocted extract of Kabran01, IEK: Infused extract of Kabran01, HEK: Hydroethanolic extract of Kabran01, N: Total number of rats in group and -: Absence of lesions

and hydroalcoholic Kabran01 at 2000 mg/kg significantly increased the relative masses of pancreas, kidney and heart of rats ($p<0.05$).

Also, histological examinations showed that the oral administration of Kabran01 at 2000 mg/kg b.wt., exerted minimal alterations at the level of the different organs observed under the microscope. In fact, Kabran01 decocted caused minimal ectasia of the heart and minimal steatosis of the liver. Its infusion and hydroalcoholic extract caused moderate pyknosis and steatosis of the liver and ectasia. The infused of Kabran01 caused minimal ectasia of the heart and pancreas, moderate hepatic pyknosis and steatosis and necrosis of the renal tubules. The hydroalcoholic extract caused moderate pyknosis and steatosis of the liver and minimal atrophy and ectasia of the pancreas and minimal dilatation of the renal tubules (Table 3).

Acute toxicity of Kabran01 at 5000 mg/kg, PO: Similar to the naked eye observations made in the previous study, acute administration of Kabran01 (5000 mg/kg, b.wt.) showed no apparent signs of suffering, disease, mortality or abnormal behaviour in the rats. Also, body weights (Fig. 2a) and water (Fig. 2b) and food (Fig. 2c) consumption of rats treated with this natural product were comparable to those of control rats.

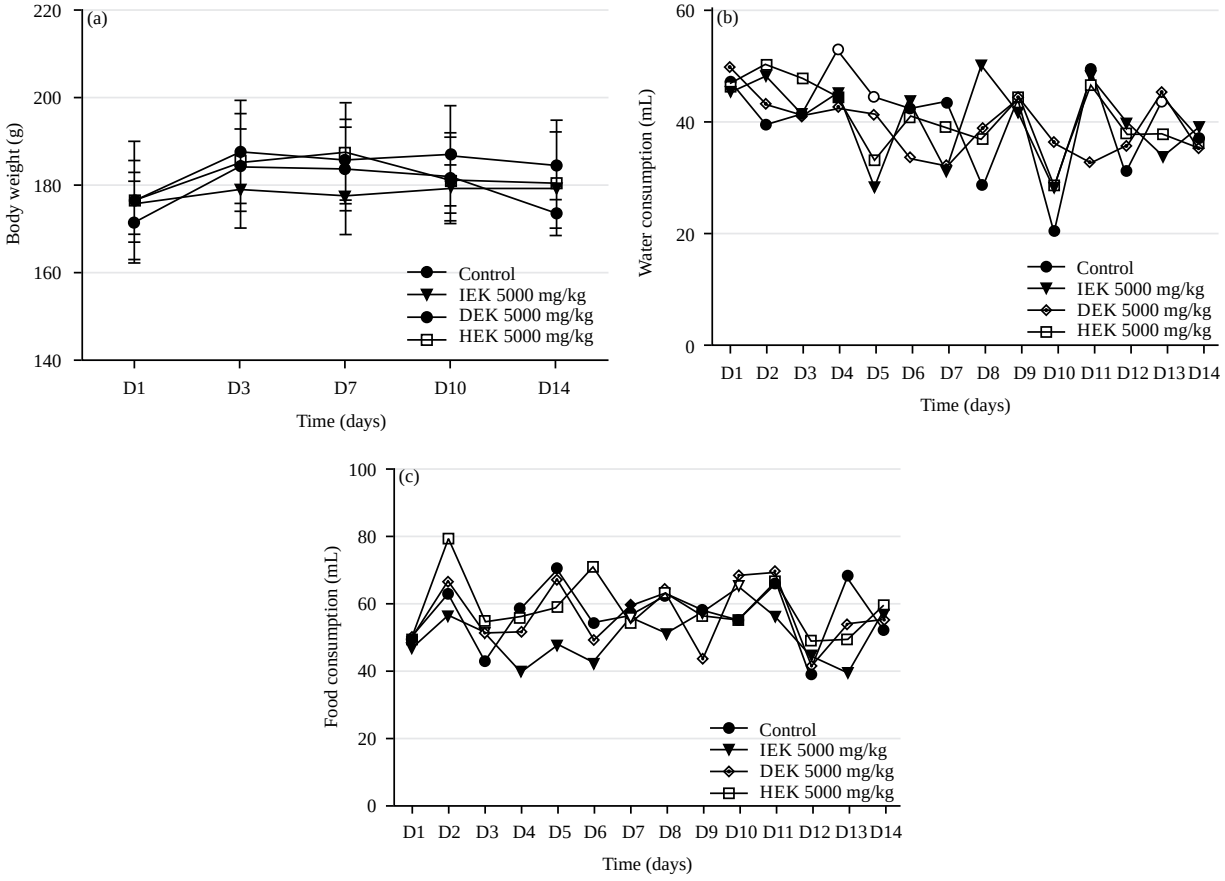

Fig. 2(a-c): Acute effect of Kabran01 recipe (5000 mg/kg, b.wt.) on clinical parameters of rats (n = 3/group), (a) Body weight of rats, (b) Water consumption and (c) Food consumption

Results are express as Mean±SEM, Statistic analysis: ANOVA followed by Bonferroni's post tests, $p<0.05$, Kabran01 has no significant influence on body weight, water and food consumption of rats

At the dose of 5000 mg/kg, blood urea and creatinine were comparable in the different groups. Also, ALT and AST were comparable in the different groups. The ionogram was comparable in the different groups. Although, the haemoglobin level decreased in the decocted, infused and hydroalcoholic rats, the differences were not significant (Table 4). The relative masses of the pancreas, kidney and heart were comparable in the different groups of treated and control rats (Table 5). Histological examinations showed that this dose of Kabran01 caused minimal and moderate alterations in the various organs. Indeed, the decocted of Kabran01 caused minimal inflammation of the heart, minimal ectasia and steatosis of the liver and moderate tubular dilatation. The infused one caused moderate hypertrophy of the heart, moderate steatosis of the liver and moderate tubular dilatation. Hydroalcoholic extract caused moderate liver steatosis and moderate renal tubular dilatation (Table 6).

Sub-acute toxicity of Kabran01 at 1000 mg/kg, PO, 28 days of rats: Subacute administration of Kabran01 at 1000 mg/kg, PO, for 28 consecutive days had no effect on body weight (Fig. 3a), water consumption (Fig. 3b) and food intake (Fig. 3c).

Oral administration of Karban01 at a dose of 1000 mg/kg for 28 days did not alter the biochemical and haematological parameters and the blood ionogram compared to control rats (Table 7). Relative organ weights were comparable in the different groups of treated and control rats (Table 8). Histological analyses of these organs showed minimal and moderate alterations in the different organs of rats treated with Kabran01. Indeed, decoction of Kabran01 caused minimal oedema and inflammation of the heart, minimal ectasia of the liver and minimal renal tubular dilatation (Fig. 4 and 5). Infusion caused moderate hypertrophy and minimal oedema of the heart. The hydroalcoholic extract induced minimal atrophy and inflammation of the pancreas (Table 9).

Table 4: Acute effect of Kabran01 at 5000 mg/kg on blood parameters of rats at day-14

	Experimental groups of rats (n = 3/group)			
Parameters	Control	DEK 5000 mg/kg	IEK 5000 mg/kg	HEK 5000 mg/kg
Biochemical				
CPK (UI/L)	105.67±3.38	111.67±5.81	119.00±4.36	109.33±4.70
Urea (mmol/L)	0.32±0.03	0.30±0.02	0.32±0.02	0.29±0.03
Creatinine (µmol/L)	5.00±0.58	5.33±0.67	4.00±0.58	4.33±0.88
Cholesterol (UI/L)	0.75±0.05	0.69±0.09	0.73±0.09	0.57±0.01
Triglycerides (UI/L)	0.88±0.05	0.93±0.11	0.98±0.11	0.66±0.15
ALT (UI/L)	27.33±3.18	29.67±0.67	36.33±6.69	18.33±5.24
AST (UI/L)	158.00±34.12	153.00±21.93	183.33±49.87	102.33±13.54
PAL (UI/L)	140.67±16.18	100.33±12.44	121.00±9.45	76.33±13.17*
Ionogram				
Natremia (mmol/L)	139.00±0.58	140.33±1.76	139.00±1.15	138.33±0.88
Kalemia (mmol/L)	4.50±0.36	4.93±0.17	4.63±0.49	4.17±0.07
Chloremia (mmol/L)	97.33±1.86	97.33±0.33	99.00±0.58	99.33±1.20
Haematological				
Erythrocytes (10^6/mm^3)	7.53±0.37	6.68±0.42	7.07±0.18	7.10±0.04
Leucocytes (10^6/mm^3)	10.65±0.72	7.75±0.94	6.17±1.65	6.50±0.07
Haemoglobin [Hg (g/dL)]	13.40±0.40	12.17±0.73	12.47±0.32	12.10±0.08
Platelets (10^3/mm^3)	872.00±34.67	578.00±138.22	808.67±114.06	539.50±125.33

Results are expressed as Mean±EMS, Statistical analysis: ANOVA followed by Tukey's Multiple Comparison Test, $p<0.05$, DEK: Decocted extract of Kabran01, IEK: Infused extract of Kabran01, HEK: Hydroethanolic extract of Kabran01, CPK: Creatine phosphokinase, ALT: Alanine Aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase and N: Number of rats in group, There was no difference between the values of the biochemical, ionographic and haematological parameters in the different experimental groups of rats

Table 5: Acute effect of Kabran01 at 5000 mg/kg on relative organ mass of rats at day-14

		Vital organs from experimental groups of rats (n = 3/group)			
Group	Rat (g)	Heart (g%)	Pancreatic (g%)	Liver (g%)	Kidneys (g%)
Control	173.70±3.20	1.42±0.14 (0.82)	1.36±0.04 (0.78)	5.64±0.12 (3.24)	1.64±0.04 (0.94)
DEK 5000 mg/kg	184.70±10.25	1.39±0.09 (0.75)	1.64±0.02 (0.89)	6.34±0.37 (3.43)	1.62±0.10 (0.88)
IEK 5000 mg/kg	179.47±5.98	1.30±0.04 (0.73)	1.43±0.13 (0.79)	5.91±0.59 (3.29)	1.60±0.08 (0.89)
HEK 5000 mg/kg	180.50±12.02	1.28±0.04 (0.71)	1.42±0.07 (0.79)	7.37±0.88 (4.06)	1.67±0.14 (0.93)

Results are expressed as Mean±EMS. Statistical analysis (ANOVA followed by Bonferroni's Multiple Comparison Test, *$p<0.05$, **$p<0.01$, ***$p<0.001$), DEK: Decocted extract of Kabran01, IEK: Infused extract of Kabran01, HEK: Hydroethanolic extract of Kabran01 and N: Number of rats in group

Table 6: Acute effect of Kabran01 at 5000 mg/kg on histology of vital organs of rats at day-14

		Experimental groups of rats (n = 3/group)			
Organs	Lesions	Control	DEK 5000 mg/kg	IEK 5000 mg/kg	HEK 5000 mg/kg
Heart	Hypertrophy (cardiomyocytes)	-		Moderate (33%)	
	Ectasia	Minimal (100%)	Minimal (33%)	Minimal (100%)	Minimal (33%)
	Oedema	-			
	Congestion	Minimal (100%)	Minimal (100%)	Minimal (100%)	Minimal (100%)
	Inflammation	-	Minimal (33%)	-	
Liver	Necrosis	Pycnosis(33%)	Pyknosis (33%)	Pyknosis (33%)	Pyknosis (33%)
	Fibrosis	-	-	-	
	Steatosis	-	Minimal (33%)	Moderate (33%)	Moderate (67%)
	Congestion	Minimal (100%)	Minimal (67%)	Minimal (100%)	Minimal (100%)
	Ectasia	-	Minimal (100%)		
	Peliosis	-			
	Inflammation	Minimal (100%)	Minimal (100%)	Moderate (33%)	Minimal (33%)
Pancreas	Necrosis	-	-	-	-
	Atrophy	-	-	-	-
	Cystic Fibrosis	-	-	-	-
	Ectasia	Minimal (100%)	Minimal (33%)	Moderate (100%)	Minimal (100%)
	Fibrosis	-	-	-	-
	Steatosis	-	-	-	-
	Hypertrophy	-	-	-	-
	Inflammation	-	-	-	-

Table 6: Continue

Organs	Lesions	Experimental groups of rats (n = 3/group) Control	DEK 5000 mg/kg	IEK 5000 mg/kg	HEK 5000 mg/kg
Kidney	Glomeruli dilatation	-	-		
	Tubules dilatation	-	Moderate (33%)	Moderate (33%)	Moderate (67%)
	Tubular necrosis	-	-	-	-
	Ectasia	-	-	-	-
	Oedema	-	-	-	-
	Congestion	-	-	-	-
	Inflammation	-	-	-	-

Microscopic lesions observed in organs from rats treated with Kabran01 are presented as a proportion (number of rats with lesions observed/total number of rats treated), Lesions are arbitrarily classified as minimal, moderate or severe according to their severity, DEK: Decocted extract of Kabran01, IEK: Infused extract of Kabran01, HEK: Hydroethanolic extract of Kabran01, N: Total number of rats in group and -: Absence of lesions

Table 7: Effect of Kabran01 at 1000 mg/kg/28 days on blood parameters of rats

Parameters	Experimental groups of rats (n = 6/group) Control	DEK 1000 mg/kg	IEK 1000 mg/kg	HEK 1000 mg/kg
Biochemical				
CPK (UI/L)	182.67±9.24	157.75±11.44	192.20±7.12	177.80±8.35
Urea (mmol/L)	0.35±0.02	0.37±0.04	0.29±0.01	0.32±0.02
Creatinine (µmol/L)	5.83±0.31	6.25±0.25	5.40±0.24	6.00±0.32
Cholesterol (UI/L)	0.72±0.03	0.77±0.02	0.68±0.03	0.67±0.03
Triglycerides (UI/L)	0.89±0.05	0.97±0.11	0.98±0.11	0.86±0.15
ALT (UI/L)	82.33±38.66	42.75±4.57	35.20±4.52	41.80±2.91
AST (UI/L)	348.33±96.74	163.75±7.96	192.40±49.12	136.00±8.32
PAL (UI/L)	226.83±21.20	213.50±5.55	193.00±18.51	179.40±18.34
Ionogram				
Natremia (mmol/L)	137.33±0.95	137.00±1.58	138.00±1.26	136.80±0.37
Kalemia (mmol/L)	4.86±0.08	4.91±0.07	4.59±0.12	4.79±0.28
Chloremia (mmol/L)	106.00±1.98	103.25±1.84	101.00±0.32	101.60±0.68
Haematological				
Erythrocytes (10^6/mm^3)	7.72±0.94	7.53±0.28	7.69±0.73	7.71±0.71
Leucocytes (10^6/mm^3)	8.46±2.56	9.78±2.88	10.29±3.94	9.47±3.03
Haemoglobin [Hg (g/dL)]	13.38±1.37	13.38±0.66	13.24±0.64	13.58±0.29
Platelets (10^3/mm^3)	825.50±367.34	1016.00±62.60	986.40±186.90	1163.20±91.78

Results are expressed as mean+/-EMS, Statistical analysis: ANOVA followed by Tukey's Multiple Comparison Test, $p<0.05$, DEK: Decocted extract of Kabran01, IEK: Infused extract of Kabran01, HEK: Hydroethanolic extract of Kabran01, CPK: Creatine phosphokinase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase and N: Number of rats in group, There was no difference between the values of the biochemical, ionographic and haematological parameters in the different experimental groups of rats

DISCUSSION

The design of new drugs for human use requires safety studies of drug candidates, mostly from local pharmacopoeias in which natural products play a major role. Toxicological assessment is carried out on laboratory to predict drug adverse reaction or drug intoxication and to provide scientific data for selecting an efficient dose in humans[8].

In the present toxicity study, rats were given acute oral doses at 2 and 5 g/kg and a subacute dose at 1 g/kg of Kabran01. The dosages was selected according to OECD 423 and 407 principles which assessing the toxicity of products already used in humans. The clinical observations showed that acute and subacute administration of Kabran01 did cause neither adverse effects nor mortality in treated rats. The LD_{50} of Kabran01 is therefore greater than 5 g/kg in rats. This finding is a relevant advantage for its use in humans. In practice, Kabran01 is used orally in folk medicine in Côte d'Ivoire at a much lower dose than 5 g/kg b.wt. This dose of a handful (about 80-150 kg) of fresh crushed plant material infused in 250 mL (1 glass of water) would logically be safe for human consumption, especially as mice are more sensitive to oral toxicity than humans[13]. However, we must take care of is routine use. The absence of signs of adverse effects in this study would not mean that there are any adverse physiological or tissue changes induced by Kabran01 in animals. In fact, it is very difficult to determine certain adverse effects in animals such as headache, dizziness and visual disturbances[14].

The analysis of blood parameters is of great scientific interest for toxicity risk assessment, as changes in the haematological system have a higher predictive value for human toxicity, when the data are translated from animal studies[15]. The toxicity studies are reassuring. Acute or

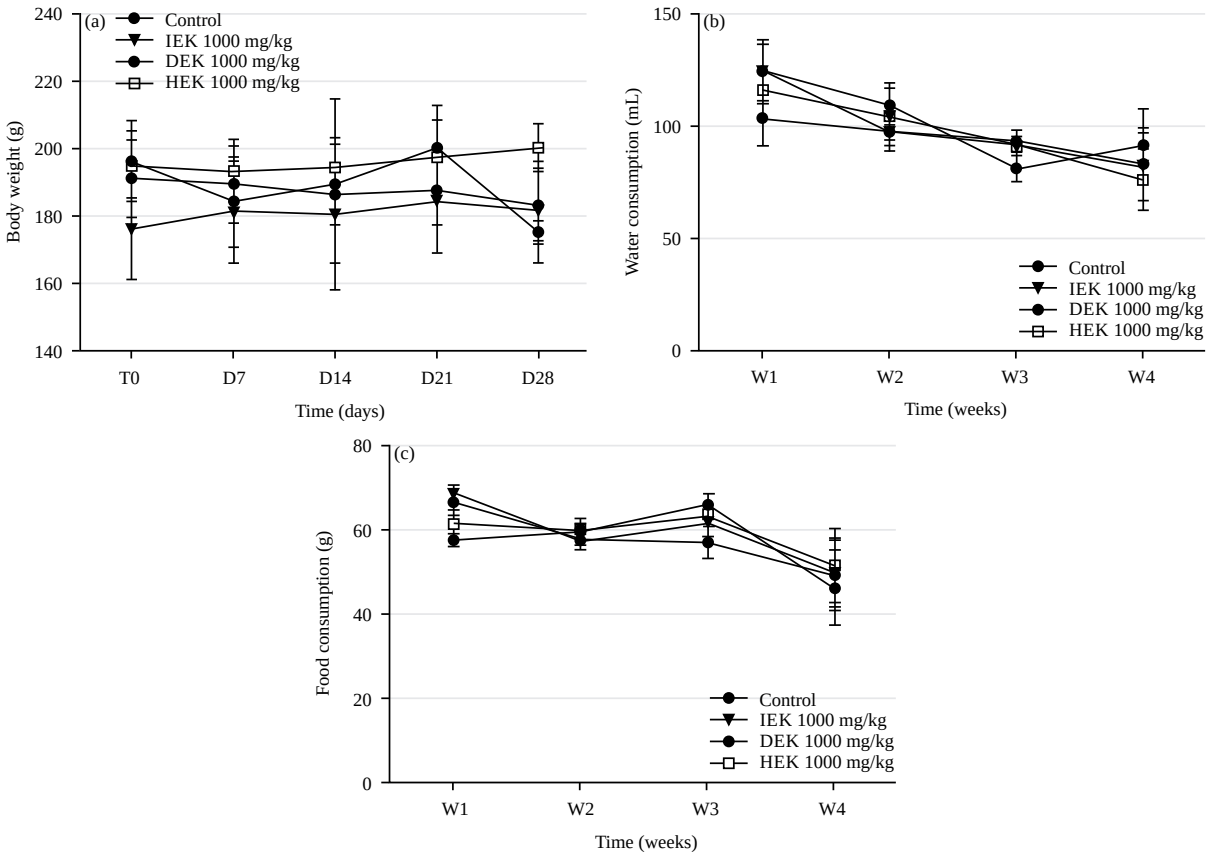

Fig. 3(a-c): Acute effect of Kabran01 recipe (1000 mg/kg/28 days) on rats (n = 3/group), (a) Body weight of rats, (b) Water consumption and (c) Food consumption

Results are express as Mean±SEM, Statistical analysis: ANOVA followed by Bonferroni's post tests, $p<0.05$. Kabran01 has no significant influence on body weight, water and food consumption of rats

Table 8: Effect of Kabran01 at 1000 mg/kg/28 days on relative organ mass of rats

		Vital organs from experimental groups of rats (n = 3/group)			
Group	Rat (g)	Heart (g%)	Pancreatic (g%)	Liver (g%)	Kidneys (g%)
Control	183.12±10.77	0.84±0.08 (0.47)	1.08±0.12 (0.61)	6.46±0.47 (3.54)	1.16±0.07 (0.64)
DEK 1000 mg/kg	175.73±3.37	0.61±0.06 (0.35)	0.55±0.09 (0.31)	5.15±0.11 (2.93)	0.89±0.03 (0.50)
IEK 1000 mg/kg	181.34±15.91	0.75±0.06 (0.42)	0.68±0.09 (0.38)	5.93±0.47 (3.29)	1.03±0.15 (0.56)
HEK 1000 mg/kg	200.42±7.25	0.79±0.07 (0.39)	0.66±0.04 (0.33)	6.04±0.20 (3.02)	1.14±0.13 (0.56)

Results are expressed as Mean±EMS, Statistical Analysis (ANOVA followed by Bonferroni's Multiple comparison Test, *p<0.05, **p<0.01, ***p<0.001), DEK: Decocted extract of Kabran01, IEK: Infused extract of Kabran01, HEK: Hydroethanolic extract of Kabran01 and N: Number of rats in group

sub-acute oral treatment with different doses of Kabran01 did not alter the biochemical and haematological parameters and the ionogram of rats. Indeed, the normal CPK level observed in treated rats would indicate good tolerance at the muscular level[16]. The lipid profile of the treated rats was maintained at a normal level, which would mean that Kabran01 does not cause cardiovascular risks[17]. The levels of transaminases (AST, ALT and PAL) and kidney parameters (urea and creatinine) were comparable to those of control rats, reflecting the preservation of liver and kidney function during chronic treatment with Kabran01[18,19]. The Kabran01 does not induce any changes in the haematological profile of treated rats. The erythrocytes, leukocytes and platelets are normal and similar to those of control rats. Such results would support the evidence for the safety of Kabran01 in animals. Similar findings have been reported in previous studies of certain medicinal plants[20]. As Kabran01 is a potential natural product with an anti-diabetic effect, it seems normal that it would normalize

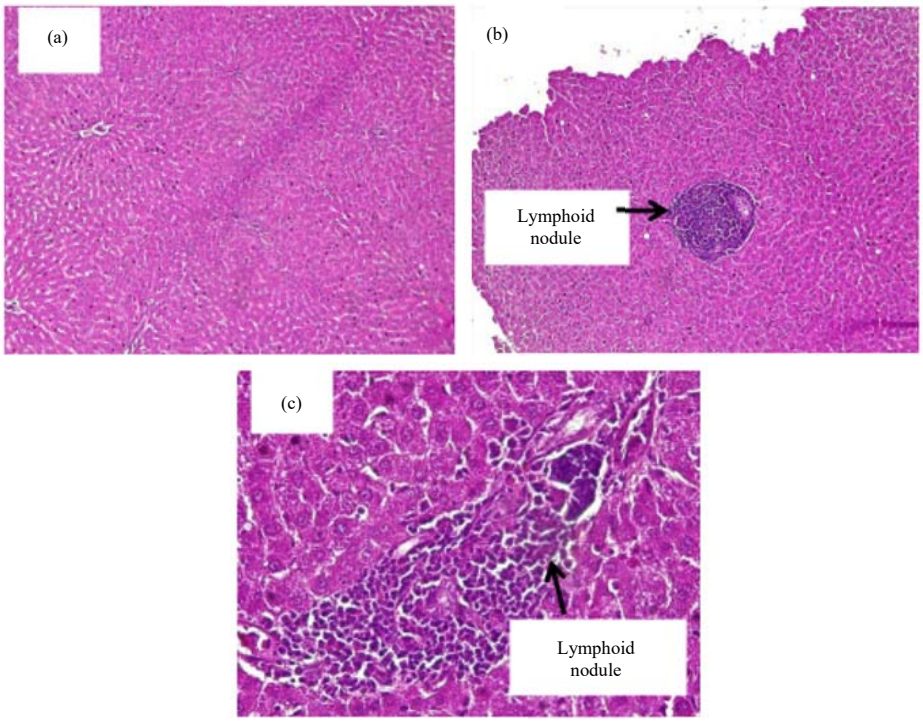

Fig. 4(a-c): Pathological analysis of rat liver after subacute treatment with Kabran01 1000 mg/kg/28 days, (a) Normal liver (H&EX100), (b) Chronic hepatitis (H&EX50) and (c) Chronic hepatitis (H&E×100)

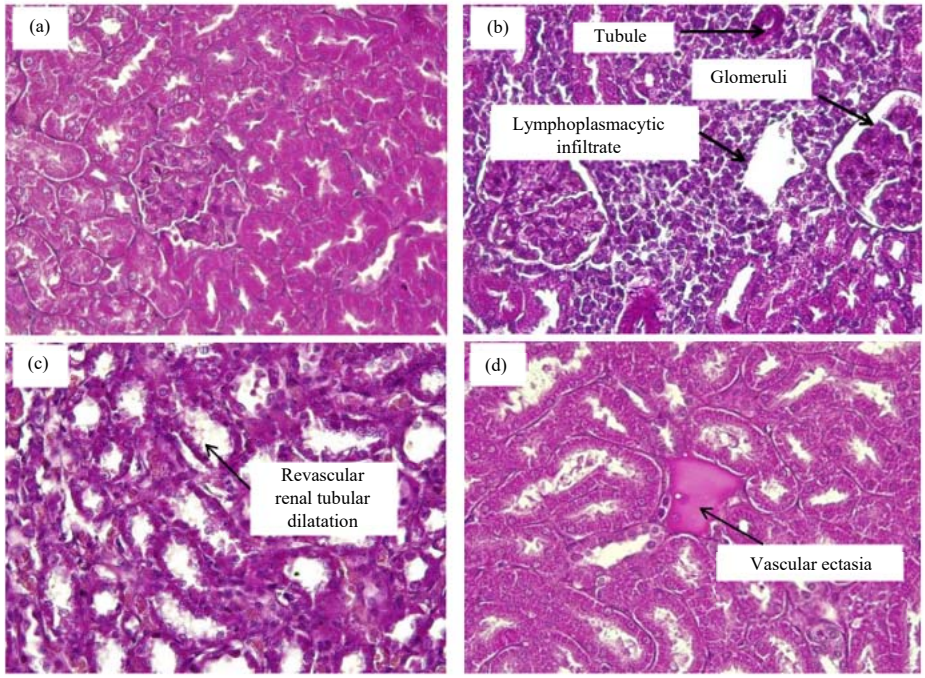

Fig. 5(a-d): Pathological analysis of the kidney of rats after subacute treatment with Kabran01 1000 mg/kg/28 days, (a) Normal kidney (H&EX400), (b) Interstitial nephritis (H&EX50), (c) Dilated renal tubules (H&EX100) and (d) Vascular ectasia between the renal tubules (H&EX400)

Table 9: Effect of Kabran01 at 1000 mg/kg/28 days on blood parameters of rats

		Experimental groups of rats (n = 6/group)			
Organs	Lesions	Control	DEK 1000 mg/kg	IEK 1000 mg/kg	HEK 1000 mg/kg
Heart	Hypertrophy (cardiomyocytes)	-		Moderate (17%)	
	Ectasia	Minimal (100%)	Minimal (67%)	Minimal (83%)	Minimal (33%)
	Oedema	-	Minimal (17%)	Minimal (83%)	
	Congestion	Minimal (100%)	Minimal (67%)	Minimal (83%)	Minimal (67%)
	Inflammation	-	Minimal (17%)	-	
Liver	Necrosis	Pycnosis (33%)	Pyknosis (17%)	Pyknosis (33%)	Pyknosis (17%)
	Fibrosis	-			
	Steatosis	Minimal (33%)	Minimal (33%)	Minimal (67%)	Minimal (17%)
	Congestion	Minimal (33%)	Minimal (50%)	Minimal (83%)	Minimal (83%)
	Ectasia	-	Minimal (33%)	Minimal (83%)	
	Peliosis	-			
	Inflammation	Minimal (100%)	Minimal (67%)	Moderate (17%)	Minimal (83%)
Pancreas	Necrosis	-	-	-	-
	Atrophy	-	-	-	Minimal (33%)
	Cystic Fibrosis	-	-	-	-
	Ectasia	Minimal (67%)	-	Minimal (83%)	Minimal (33%)
	Fibrosis	-	-	-	-
	Steatosis	Minimal (67%)	-	-	-
	Hypertrophy	-	-	-	-
	Inflammation	-	-	-	Minimal (17%)
Kidney	Glomeruli dilatation	-	-	-	-
	Tubules dilatation	-	Minimal (50%)	-	-
	Tubular necrosis	Minimal (67%)	-	Moderate (33%)	Minimal (17%)
	Ectasia	Minimal (67%)	Minimal (17%)	-	Minimal (17%)
	Oedema	Minimal (67%)	-	-	-
	Congestion	Minimal (67%)	Minimal (50%)	Minimal (17%)	Minimal (33%)
	Inflammation	Minimal (67%)	Minimal (33%)	Moderate (33%)	-

Microscopic lesions observed in organs from rats treated with Kabran01 are presented as a proportion (number of rats with lesions observed/total number of rats treated), lesions are arbitrarily classified as minimal, moderate or severe according to their severity, DEK: Decocted extract of Kabran01, IEK: Infused extract of Kabran01, HEK: Hydroethanolic extract of Kabran01, N: Total number of rats in group and -: Absence of lesions

blood biological parameters so that the body could regulate blood sugar levels effectively by fighting against insulin resistance. The Kabran01 improve quality of live than classic antidiabetic (unpublished). Despite the good clinical and biological tolerance of high and repeated dosages of Kabran01, microscopy examination seems to confirm this good tolerance for heart, pancreas and kidney.

Nevertheless, microscopic examinations of organ sections from rats treated with the various acute and sub-acute oral treatments of the different Kabran01 preparations overall showed minimal to moderate alterations of their heart, liver, pancreas and kidneys. These minimal alterations were ectasia, inflammation, oedema of the heart and hypertrophy of cardiomyocytes. These treatments at high dose exhibited at most a moderate hepatic ectasia, pyknosis and steatosis (Fig. 4), also minimal atrophy, inflammation and ectasia of the pancreas. In the kidney, Kabran01 high doses caused minimal renal tubular dilatation and necrosis. This renal tubular dilatation was found to be moderate during subacute administration (Fig. 5).

Although, histopathological analyses showed minor alterations, justifying the good tolerance of Kabran01. At the therapeutic doses, these effects must be more monitored. Also, these effects at the tissue level in animals could not, directly, be extrapolated to humans[13]. Nevertheless, the evaluation of the adverse effects of sub-chronic and chronic administration in laboratory may be more relevant to determine the overall toxicity of the plant preparation, especially as the preparation Kabran01 is used in the long-term treatment of diabetes. To be sure and secure, chronic therapeutic dose need to be monitored.

CONCLUSION

At the doses consumed empirically in traditional ivorian medicine, Kabran01 appears to be relatively non-toxic, because its higher doses could induce minor heart, liver, pancreas and kidney toxicity. So the use of Kabran01 at therapeutic dose must be monitored to better assess its risk-benefit balance. The Kabran01 recipe seems better drug candidate for clinical trial really live.

SIGNIFICANCE STATEMENT

The aim of the present study was to provide scientific evidence of the tolerability of the herbal preparation Kabran01 at high acute doses of 2000 and 5000 mg/kg and at sub-acute doses of 1000 mg/kg in rats. The results showed that Kabran01 had no adverse effects on general behaviour and no mortality in rats, justifying its long-standing use by the Ivorian population as an anti-diabetic drug. However, microscopic observations revealed minor damage to vital organs in these rats. Prospectively, the therapeutic dose of Kabran01 must be determined for its controlled and efficient use in the treatment of diabetes in humans.

REFERENCES

1. Petrovska, B.B., 2012. Historical review of medicinal plants' usage. Pharmacogn. Rev., 6: 1-5.
2. Fokunang, C.N., V. Ndikum, O.Y. Tabi, R.B. Jiofack and B. Ngameni *et al*., 2011. Traditional medicine: Past, present and future research and development prospects and integration in the national health system of Cameroon. Afr. J. Tradit. Complementary Altern. Med., 8: 284-295.
3. Pengpid, S. and K. Peltzer, 2019. Use of traditional medicines and traditional practitioners by children in Indonesia: Findings from a national population survey in 2014-2015. J. Multidiscip. Healthcare, 12: 291-298.
4. Bi, F.H.T., G.M. Irie, K. N'Gaman and C.H.B. Mahou, 2008. Study of some therapeutic plants used to treat arterial hypertension and diabetes: Two emergent diseases in Côte d'Ivoire [In French]. Sci. Nature, 5: 39-48.
5. Beutler, J.A., 2009. Natural products as a foundation for drug discovery. Curr. Protoc. Pharmacol., Vol. 46. 10.1002/0471141755.ph0911s46.
6. Atanasov, A.G., S.B. Zotchev, V.M. Dirsch, I.N.P.S.T. and C.T. Supuran, 2021. Natural products in drug discovery: Advances and opportunities. Nat. Rev. Drug Discovery, 20: 200-216.
7. Villanueva, J.R., J.M. Esteban and L.R. Villanueva, 2018. Pharmacological activities of phytomedicines: A challenge horizon for rational knowledge. Challenges, Vol. 9. 10.3390/challe9010015.
8. Jităreanu, A., A. Trifan, M. Vieriu, I.C. Caba, I. Mâru and L. Agoroaei, 2023. Current trends in toxicity assessment of herbal medicines: A narrative review. Processes, Vol. 11. 10.3390/pr11010083.
9. Ouedraogo, G.G., S. Ilboudo, S. Ouedraogo, J.C.R.P. Ouedraogo and G.D. Somda *et al*., 2020. Acute and subacute oral toxicity studies and anti-sickling activity assessment of FACA® syrup. J. Drug Delivery Ther., 10: 40-50.
10. Efferth, T. and B. Kaina, 2011. Toxicities by herbal medicines with emphasis to traditional Chinese medicine. Curr. Drug Metab., 12: 989-996.
11. Kiliś-Pstrusińska, K. and A. Wiela-Hojeńska, 2021. Nephrotoxicity of herbal products in Europe-A review of an underestimated problem. Int. J. Mol. Sci., Vol. 22. 10.3390/ijms22084132.
12. Kiani, A.K., D. Pheby, G. Henehan, R. Brown and P. Sieving *et al*., 2022. Ethical considerations regarding animal experimentation. J. Preventive Med. Hyg., 63: E255-E266.
13. Vocci, F. and T. Farber, 1988. Extrapolation of animal toxicity data to man. Regul. Toxicol. Pharmacol., 8: 389-398.
14. Akhtar, A., 2015. The flaws and human harms of animal experimentation. Cambridge Q. Healthcare Ethics, 24: 407-419.
15. Olson, H., G. Betton, D. Robinson, K. Thomas and A. Monro *et al*., 2000. Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul. Toxicol. Pharmacol., 32: 56-67.
16. Bhattacharyya, K., S. Phaujdar, R. Sarkar and O.S. Mullick, 2018. Serum creatine phosphokinase: A probable marker of severity in organophosphorus poisoning. Toxicol. Int., 18: 117-123.
17. Yin, W., E. Carballo-Jane, D.G. McLaren, V.H. Mendoza and K. Gagen *et al*., 2011. Plasma lipid profiling across species for the identification of optimal animal models of human dyslipidemia. J. Lipid Res., 53: 51-65.
18. Aithal, G.P., N. Guha, J. Fallowfield, L. Castera and A.P. Jackson, 2012. Biomarkers in liver disease: Emerging methods and potential applications. Int. J. Hepatol., Vol. 2012. 10.1155/2012/437508.
19. Jo, S.K., J. Yang, S.M. Hwang, M.S. Lee and S.H. Park, 2019. Role of biomarkers as predictors of acute kidney injury and mortality in decompensated cirrhosis. Sci. Rep., Vol. 9. 10.1038/s41598-019-51053-8.
20. Madrigal-Santillan, E., E. Madrigal-Bujaidar, I. Alvarez-Gonzalez, M.T. Sumaya-Martinez and J. Gutierrez-Salinas *et al*., 2014. Review of natural products with hepatoprotective effects. World J. Gastroenterol., 20: 14787-14804.