Research Article

Effects of dichloromethanic extract of *Piliostigma reticulatum* D.C. (Hochst) stem bark on some biochemical parameters of Wistar albino rats.

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ABSTRACT

***Piliostigma reticulatum* is a medicinal plant used in traditional treatment of digestive disorders such as diarrhoea in Côte d'Ivoire. In order to check its safety, the dichloromethanic extract, the most active extract, obtained from hydro-ethanol extract used in traditional environment, was administered to three groups of rats at doses of 125, 250 and 500 mg/kg body weight. The control group received distilled water. The delivery time was 28 days. The blood collected each week allowed the determination of some biochemical parameters. The extract resulted at all doses, the increase of serum markers of kidney, and at the doses 250 and 500 mg/kg body weight increased the values of transaminases and bilirubin. The dose 500 mg/kg body weight caused an increase in serum cholesterol, total protein and hypoglycaemia. Only triglyceride levels were not affected at all doses. All these disturbances observed are reversible. Moreover, the extract does not induce delayed toxic effects.**

KEYWORDS: *Piliostigma reticulatum*, dichloromethanic extract, biochemical parameters, subacute toxicity, rat.

INTRODUCTION

The use of medicinal plants is a popular practice around the world. So, the world health organization recommended to use herbal medicines whose safety, efficiency and quality are guaranteed [30]. *Piliostigma reticulatum* is one of the medicinal plants of Côte d'Ivoire [3].This medicinal plant is used for the treatment of several diseases such as colic, hemorrhoids and particularly diarrhoea [10,36]. It is a tree measuringf 8 to 10 m high, bole rarely straight, sometimes bushy rejection by strain, with a rounded, bushy top. Its bark is deeply fissured and cracked, sometimes ferruginous gray, with a pink, fibrous slash turning brown. The leaves

are alternate, leathery, couplets, and hairless below. They are heavily lobed with rounded lobes or corner. The fruit is a woody pod, flat, hairless, sometimes twisted and cracked[6].

Several studies to verify the pharmacological activities were conducted. The leaves of *Piliostigma reticulatum* have antibacterial and antimicrobial properties [4]. The leaves have also sedative and anticonvulsant [8]. The ethanolic extract of the leaves of Piliostigma reticulatum has antimalarial activity in mice [23]. The hydro--ethanolic extract of *Piliostigma reticulatum* stem bark at doses 250, 500 and 1000 mg / kg body weight reduced diarrhoeic feces[10]. The Bioguide splitting of hydro-ethanolic extract showed that dichloromethanic extract was the most active by its high inhibition of diarrhoeic faeces and gastrointestinal mobility induce[9]. These authors also showed that LD_{50} of this extract was estimated to more than 5000 mg / kg body weight.

Given these multiple therapeutic properties, the safety assessment of hydro-ethanolic extract administered orally for 28 days, revealed that it has no toxic effect on liver and kidneys at the doses 250, 500 and 1000 mg / kg body weight [20]. The aim of this study is to evaluate the safety of dichloromethanic extract through its effect on some biochemical parameters.

MATERIAL AND METHODS

Plant material

Piliostigma reticulatum stem barks has been collected in January 2013 in Kadjabo (Dimbokro) located 240 Km from Abidjan. The identification of the species was confirmed by National Floristic Center of Abidjan. A sample is deposited under number 18033.

Preparation of the dichloromethanic extract of *Piliostigma reticulatum* stem bark

We dissolved 2.5 g of hydro-ethanolic extract powder in 100 mL of distilled water. The mixture was added to 100 mL of heptane. After stirring and settling in a separating funnel, the heptanic phase is separated from the aqueous phase. 100 ml of dichloromethane are added to the aqueous layer. Agitation and settling allow to separate the aqueous phase and the dichloromethanic one. And then the dichloromethanic phase is evaporated under vacuum at 50 ° C and then dried in an oven at 45 ° C for 14 hours. The resulting powder is the dichloromethanic extract[7,9]. It weighs about 0.276 g representing a yield of 11.03%.

Animal

Rats of the species *Rattus norvegicus* wistar strain aged 4 to 6 weeks weighing between 97 and 108 g were used for the test. All animals were subjected to a temperature of 25 ± 2 ° C with alternating 12 hours of light and 12 hours dark. They were fed with pellets of FACI® and had tap water at will without discontinuity in baby bottles. All the experiments with the animals was performed according to the ethical guidelines of OCED [29].

Subacute toxicity study

The study of subacute toxicity was conducted according to the guideline 407 concerning the oral toxicity Repeated dose for 28 days in rodents [28].

Sixty rats were divided into six groups of 10 rats including 5 males and 5 females with four test groups and two control groups. Three doses are prepared in accordance with previous studies [9]. The rats of groups 2, 3 and 4 received dichloromethanic extract respectively at doses125, 250 and 500 mg / kg of body weight. Those of groups 1 and 5 received distilled water while the group 6 received 500 mg / kg body weight of the

extract. All the animals are individually marked. The different treatments were administered orally at a rate of 2 ml / 100 g body weight. The groups 5 and 6 lots were used for the study of reversibility, persistence, or delayed occurrence of toxic effects for 14 days after stopping treatment.

Blood samples

The day of sampling, the animals are fasted for ten hours without food but with water at will [28]. The animals are anesthetized with ether, the blood is collected in the morning by the incision technique to 5 mm from the tip of the tail which is previously disinfected with alcohol 96 ° [21]. Blood is collected in dry tubes. These samples are taken in all rats one day before the beginning of the extract administration, and weekly in the first four groups. Finally, 14 days after cessation of the treatment, blood in the satellite groups of rats is taken..

Determination of biochemical parameters

Glycaemia is determined directly from whole blood using a brand Accu-Chek® (Roche Diagnostics) according to glucose oxidase method [35]. Then, the blood contained in each dry tube is centrifuged at 3000 revolutions / minute for 5 minutes and the obtained serum was used for the determination of other biochemical parameters. Alanine transferase (ALT) and aspartate transferase (AST) have been determined by the kinetic method [15]. The determination of serum total cholesterol and urea was perform by the enzymatic method [5,16,34]. Creatinine is determinated thanks to the colorimetric method [11]. Total proteins and triglycerides were also assayed by the colorimetric method [12]. Bilirubin were measured by the diazo method [24].

Statistical analysis

The values are presented as the mean followed by the standard error on the mean (SEM). Comparisons of averages are performed relative to the control, with the repeated measures ANOVA with varied, followed by post-hoc Bonferroni test. The differences are called significant for p less than 0.05. Values followed by letters indicate increases while the asterisk denote decreases. These tests were performed using Graphpad version 5.0 software was used to process the data obtained.

RESULTS

Prior to the administration of the extract, all the experiment animals had values of the parameters studied nearly equal (Table 1).

	Doses (mg/kg b.w.)			
	0	**125**	**250**	**500**
ALT (UI/L)	10.00± 0.51	11.36± 0.57	11.57±1.13	11.93± 0.75
AST (UI/L)	31.56± 1.02	30.20± 3.51	30.44± 2.35	29.67± 3.19
Urea (g/L)	2.59± 0.18	3.38± 0.27	3.02± 0.31	3.20± 0.30
Creatinine (g/L)	5.97± 0.38	6.56± 0.23	6.29± 0.27	6.49± 0.37
Total Bilirubin(g/L)	3.09± 0.42	3.68± 0.44	3.59± 0.48	3.42± 0.43
Direct bilirubin (g/L)	1.07± 0.13	1.26± 0.14	1.24± 0.18	1.16± 0.13
Total proteins (g/L)	78.32± 4.43	93.74± 4.54	88.18± 3.45	92.69± 6.88
Cholesterol (g/L)	1,19± 0.15	1.23± 0.13	1.39± 0.12	1.29± 0.18
Triglycerides (g/L)	0.82± 0.14	0.97± 0.13	1.03± 0.16	0,81± 0,16
Glycaemia (mg/dL)	99.40± 2.69	98.40± 2.24	95.80± 1.79	93,70± 2,59

Table 1. Values of biochemical parameters of rats before the administration of the extract
n = 10 animals in each group. The comparisons are made between the control group and the treated groups. 0: .lot treated with distilled water, b.w.: body weight.

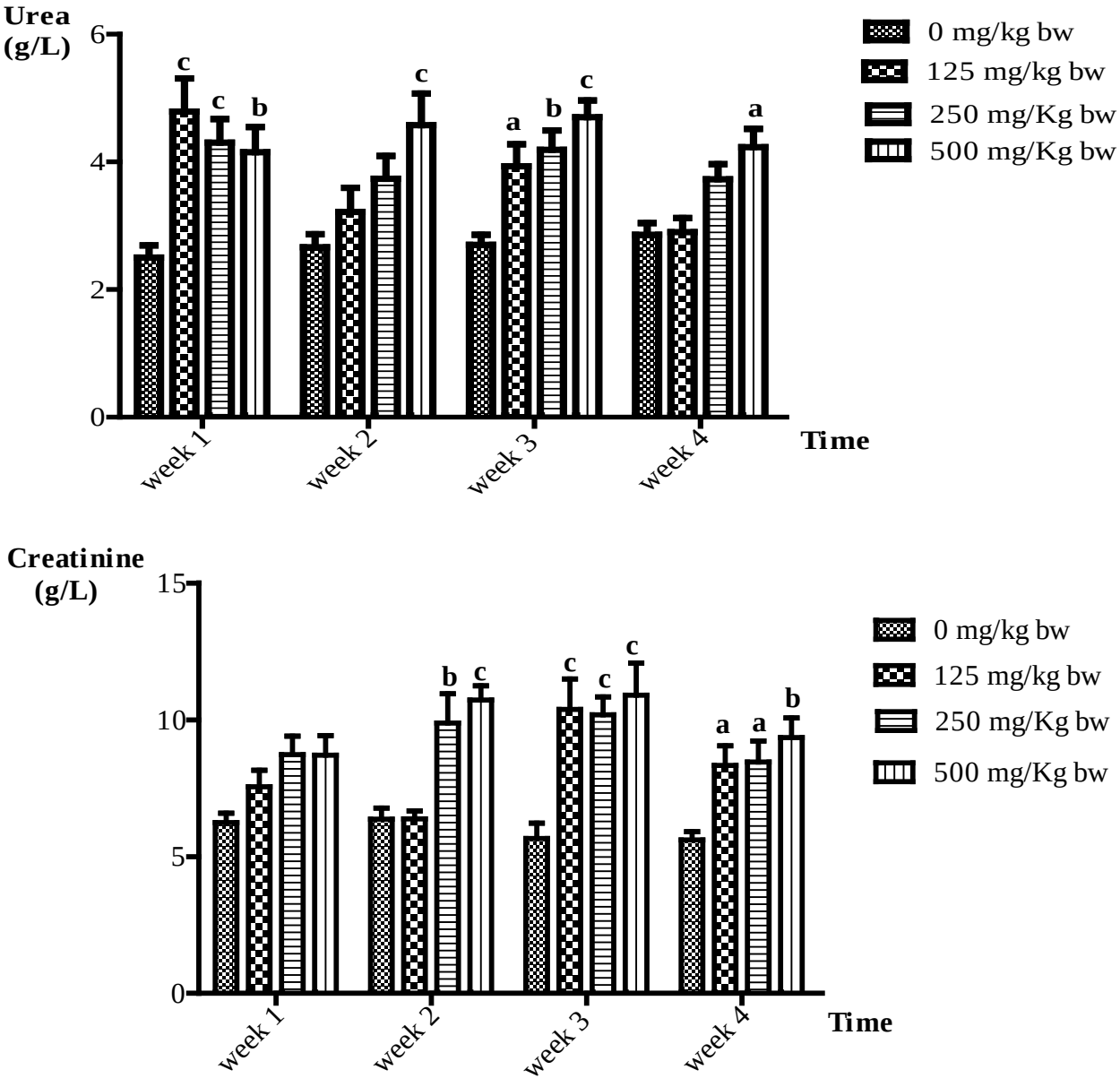

Figure 1A, 1 B. Effect of dichloromethanic extract on serum markers of kidney. A: urea; B: creatinine; a = $p<0.05$; b = $p< 0.01$; c = $p<0.001$; n = 10 animals in each group. The comparisons are made between the control group and the treated groups. 0: group treated with distilled water; b.w.: body weight.

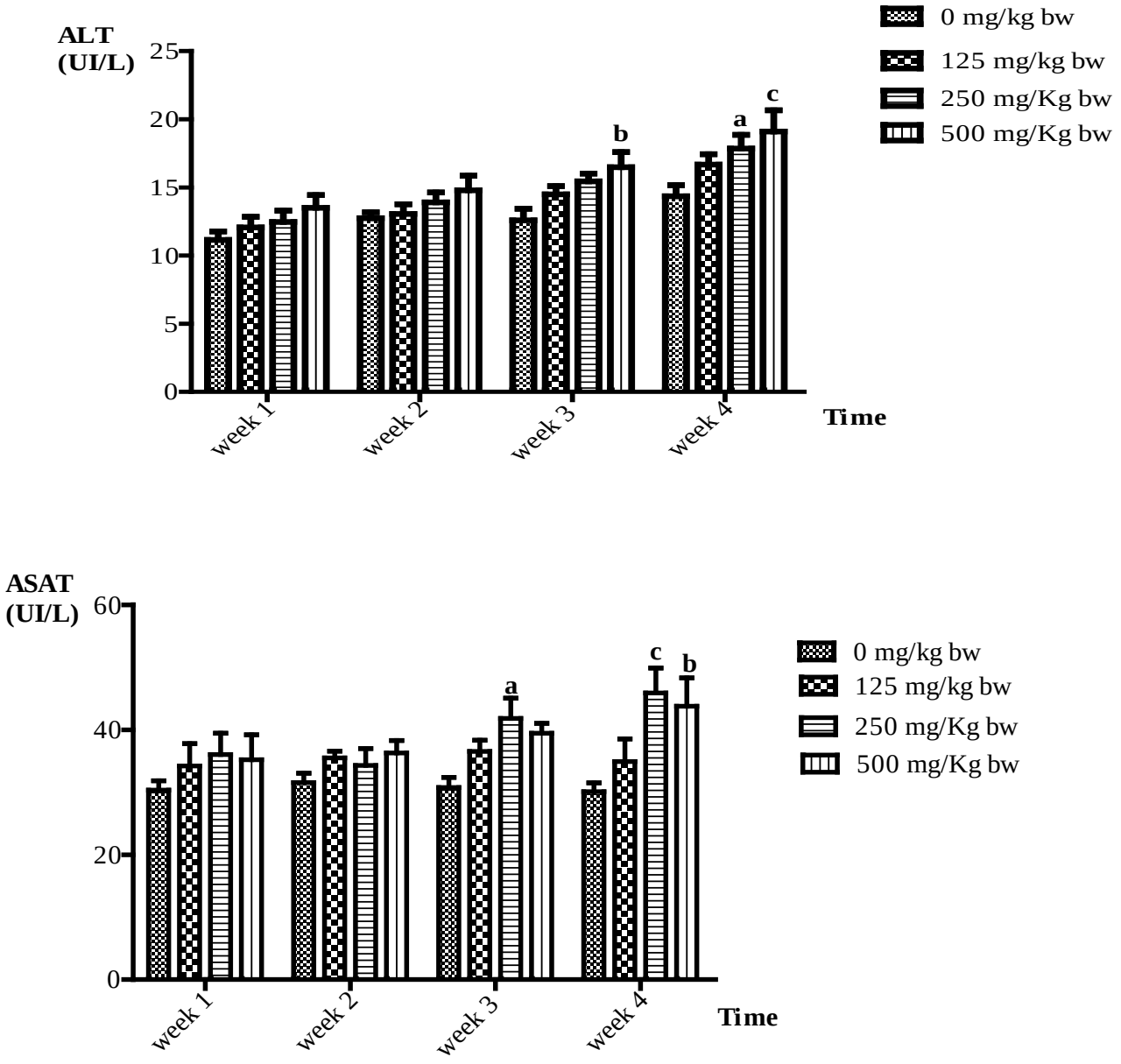

Figure 2A and 2 B. Effect of dichloromethanic extract on serum markers of liver. A: ALT; B: AST; a = p<0.05; b = p< 0.01; c = p<0.001; n = 10 animals in each group. The comparisons are made between the control group and the treated groups. 0: .lot treated with distilled water, b.w.: body weight.

Urea and creatinine levels were significantly increased at all doses respectively during the four weeks (Figure 1A) and from the second week of administration of the extract (Figure 1B).

In the third week, the dichloromethanic extract resulted in an increase in ALT (Figure 2A) and AST (Figure 2B). This increase occurred at the dose 250 mg / kg body weight ($p < 0.05$) and 500 mg / kg body weight ($p < 0.01$) and remained at the fourth week . Under the influence of the extract, the rats which received the doses 250 and 500 mg / kg body weight presented an increase of serum total

bilirubin (Table 2). These hyperbilirubinemias which began in the second week, persisted in the third week before disappearing in the fourth week. In addition, direct bilirubin of rats at the dose 500 mg / kg body weight experienced a transient significant increase ($p < 0.05$) in the third week (Table 2). In the group treated with 500 mg / kg body weight, the increase of serum cholesterol occurred in the second week, and an increase of the total proteins content the third and fourth weeks. At the same dose, hypoglycaemia is recorded from the first week to the third week. Throughout the period of the administration of the extract, the serum triglycerides in all the treated groups have not undergone major changes compared to the control group (Table 2).

Doses(mg/kg b.w.)		week 1	week 2	week 3	week 4
Total bilirubin (g/L)	0	2.60±0.33	2.59±0.3106	2.57±0.37	2.88±0.36
	125	4.06±0.37	3.36±0.36	3.70±0.37	4.133±0.39
	250	4.00±0.54	**4.26±0.41[a]**	3.99±0.49	4.14±0.55
	500	3.70±0.34	**4.40±0.21[b]**	**4.75±0.38[c]**	3.94±0.44
Direct bilirubin (g/L)	0	0.89±0.12	0.90±0.11	0.88±0.12	0.99±0.13
	125	1.42±0.16	1.16±0.12	1.29±0.13	1.43±0.19
	250	1.40±0.19	1.13±0.12	**1.26±0.16[a]**	1.42±0.19
	500	1.30±0.13	1.06±0.11	**1.18±0.12[a]**	1.332±0.15
Total proteins (g/L)	0	81.32±4.30	88.25±5.29	82.78±4.25	82.96±5.34
	125	93.65±3.10	91.36±2.48	93.37±4.16	89.51±3.36
	250	91.22±3.12	98.85±9.83	101.2±11.85	99.54±9.99
	500	99.30±9.41	96.90±8.18	**113.4±7.10[b]**	**105.5±6.22[a]**
Cholesterol (g/L)	0	1.96±0.24	1.18±0.19	2.15±0.19	1.85±0.13
	125	1.36±0.13	1.37±0.11	2.40±0.13	1.58±0.18
	250	1.71±0.13	1.60±0.11	2.13±0.13	1.68±0.18
	500	1.70±0.23	**2.22±0.62[b]**	2.69±0.35	1.66±0.16
Triglycerides (g/L)	0	0.87±0.13	0.77±0.02	0.64±0.09	0.63±0.09
	125	1.25±0.27	0.84±0.08	0.33±0.019	0.46±0.06
	250	1.24±0.27	0.82±0.08	0.42±0.05	0.41±0.06
	500	1.08±0.35	0.90±0.093	0.53±0.06	0.54±0.07
Glycaemia (mg/dL)	0	101.5±3.78	95.10±4.01	103.3±3.46	103.2±3.92
	125	103.8±4.39	95.80±6.59	104.3±5.26	94.20±4.31

	250	87.00±3.71	88.90±6.59	91.50±3.70	93.00±3.45
	500	**78.30±3.23*** **	**78.40±2.87***	**85.90±5.27***	90.70±3.63

Table 2. Effect of dichloromethanic extract of *Piliostigma reticulatum* stem bark on some biochemical parameters

n = 10 animals in each group. The comparisons are made between the control group and the treated groups, 0: .lot treated with distilled water, b.w.: body weight. For the same period and for a parameter: * = p <0.05, *** = p <0.001, a = p <0.05; b = p <0.01; c = p <0.001.

Upon discontinuation of treatment, the transaminase ALT, AST, urea, creatinine and total proteins levels increased significantly at the dose 500 mg / kg body weight (Table 3) .However, two weeks after the end of the administration of the extract, all these parameters are normalized. Furthermore, the parameters were not significantly changed during the administration of the extract were unchanged after the interruption of the force-feedings (Table 3).

	week 4		**week 6**	
	Doses (mg/kg b.w.))		**Doses (mg/kg b.w.)**	
	0	500	0	500
ALT (UI/L)	14.35±0.81	**19.09±1.57^{c}**	12.85±1.83	12.24±1.74
AST (UI/L)	30.07±1.43	**43.78±4.53^{b}**	33.05±3.11	37.49±2.20
Urea (g/L)	2.86±0.19	**4.23±0.29^{a}**	2.90±0.40	3.00±0.21
Creatinine (g/L)	5.61±0.31	**9.35±0.71^{b}**	5.62±0.39	5.92±0.31
Bilirubin (g/L)	2.88±0.36	4.13±0.39	2.60±0.41	3.58±0.56
Direct bilirubin (g/L)	0.99±0.13	1.43±0.13	0.91±0.14	1.33±0.17
total Protéins (g/L)	82.96±5.34	**105.5±6.22^{b}**	83.76±5.30	95.12±4.10
Cholesterol (g/L)	1.85±0.13	1,66±0.17	1.54±0.50	1.32±0.36
Triglycerides (g/L)	0.63±0.10	0,54±0.07	1.26±0.17	1.39±0.28
Glycaemia (mg/dL)	103.2±3.92	90,70±3.63	102.3±3.87	97.90±5.77

Table 3. Values of biochemical parameters of satellites rats after stopping treatment

n = 10 animals in each group. The comparisons are made between control group and treated groups, 0: .lot treated with distilled water, b.w.: body weight. For the same period and for a parameter: a = p <0.05; b = p <0.01; c = p<0.001.

DISCUSSION

The nearly equal values of all the studied parameters, obtained before the administration of the dichloromethanic extract of *Piliostigma reticulatum* stem barks assume that animals used for experiments are in the same physiological state before the experiment. This allows a better assessment of the possible changes that will take place.

The dosages of transaminases and bilirubin tests are generally used for the assessment of the liver function. The dichloromethanic extract resulted in an increased of transaminases ALT and AST at the third and fourth week at the doses 250 and 500 mg / kg body weight. Another authors observed an increase of the ALT serum concentration in treated rats with the aqueous extract of the stem bark of *Spondias mombin* for 28 days [14]. The serum AST increased also in rats treated with the latex of *Calotropis procera* [22]. But, these results are different to those obtained with the hydro-ethanolic extract of the stem bark of *Piliostigma reticulatum*, which on an identical study did not affect the concentrations of serum transaminases [20]. The transaminases are important enzymes in the metabolic activity of cells. Their increase implies deteriorations, particularly in the liver [18]. So,the dichloromethanic extract would be hepatotoxic. In addition, the serum AST gives informations about the status of skeletal muscle and myocardium [31]. Therefore, the dichloromethanic extract would cause heart tissue or skeletal muscle injuries. As regards the levels of serum total bilirubin, they increased at the second week in rats at doses 250 and 500 mg / kg body weight and at the third week only in those treated at 500 mg / kg body weight. In addition, an increase in direct bilirubin occurred at the third week in the rats which received 500 mg / kg body weight of the extract. However, the hydro-ethanolic extract did not disrupted the serum bilirubin [20]. Authors noted that the aqueous extract of *Cochlospermum planchonii* rhizomes at the dose 50 mg / kg body weight caused a decrease in serum bilirubin during an administration period between 1 and 5 days, before raising in the period between 10 to 15 days [2]. It is reported that haemolysis of erythrocytes releases heme which is converted into biliverdin, then into prehepatic bilirubin. By the portal vein, it happens to the liver where it is transformed completely into bilirubin when the liver functions are normal. Moreover, the increase of serum bilirubin can be due to a malfunction of the kidneys about the excretion of bilirubin excess or a liver failure to combine it effectively for excretion [33]. The increase of direct bilirubin implies a lower performance liver and kidney in rats treated with the dose 500 mg / kg body weight.

Creatinine and urea are markers of renal function. An increase of their concentrationsin the serum means a renal dysfunction [33]. Dichloromethanic extract induced the increase of the serum creatinine concentration at the doses 250 and 500 mg / kg body weight from the second week until the end of the force-feedings. The third week was also marked by an increase of serum creatinine concentrations of rats at a dose 125 mg / kg body weight. Daily administration of *Piliostigma reticulatum* hydro-ethanolic extract and the latex of *Calotropis procera* for 28 days, also both increased

creatinine levels [20,22]. Regarding uremia, the extract resulted in the first week an increase of serum levels at all doses of the extract with persistence until the end of the force-feedings for the rats treated at the dose 500 mg / kg body weight. These results are like those obtained with the ethanolic extract of *Ageratum conyzoides* leaves whom doses between 500 and 1000 mg / kg body weight caused hyperuremia in rats [1]. However, it is different of that one obtained with hydro-ethanolic extract of *Piliostigma reticulatum* that did not alter uremia in rats at doses between 250 and 1000 mg / kg body weight [20]. The chances are that dichloromethanic extract has kidneys toxicity. As for glycaemia, caused at the first week, hypoglycaemia in rats treated with doses 250 and 500 mg / kg body weight. This hypoglycaemia persisted until the third week in rats which received the dose 500 mg / kg body weight. In a similar study, the hypoglycaemic effect was also observed at the dose 1000 mg / kg body weight of the hydro-ethanolic extract [20]. But the daily administration of *Sacoglottis gabonensis* aqueous extract for 28 days at the doses 3.5, 35 and 350 mg / kg body weight had no effect on glycaemia of the rats [19]. Glucose can be stored in the liver or muscle as glycogen which is degraded into glucose and distributed to the tissues which need it. An abnormality of the enzymes involved in this metabolism results in glycogen overload in the liver leading to hypoglycaemia [33].

The extract could therefore affect this mechanism. Authors have shown that polyphenols have hypoglycaemic effects [25,26]. These compounds were identified in the dichloromethanic extract [9]. So, the induced hypoglycemia can be due to the presence of polyphenols, which in high doses have disturbed glucose metabolism in rats.

Dichloromethanic extract had no effect on serum triglycerides while it caused an increase of serum cholesterol at the second week in rats subjected to the dose 500 mg / kg body weight. This fact is similar to that observed when hydro-ethanolic extract of *Piliostigma reticulatum* stem bark was administered daily for 28 days. This extract at the dose 1000 mg / kg body weight resulted in hypercholesterolemia without the triglycerides rate is changed [20]. The elevation of serum cholesterol and triglycerides is a major risk factor for cardiovascular disease [2]. The extract disrupted fat metabolism and accumulation of serum cholesterol. It would promote the occurrence of cardiovascular disease.

Dichloromethanic extract caused increase of serum total proteins at the last two weeks of the experiment. This result is contrary with *Sacoglottis gabonensis* aqueous extract. It has no effect on serum total proteins in rats subjected to administration of the extract orally for 28 days [19]. Similarly, *Piliostigma reticulatum* hydro-ethanolic extract has no effect on total protein [20]. Plasma proteins are contribute for the blood buffering capacity [17,32]. In addition, the colloidal osmotic pressure due to plasma proteins maintains the fluid passage by osmosis interstitial spaces to blood [17]. Therefore the dichloromethane extract thus increase the colloidal pressure which would lead to a turgor capillaries.

After stopping the administration of the extract, the values of all the biochemical parameters become normal. This reflects that the recorded disturbances are reversible, so the effects of the extracts are eliminated by the organism of the rat.

CONCLUSION

It appears about this study that the repeated administration of the dichloromethanic extract of *Piliostigma reticulatum* stem bark, caused nephrotoxicity at all doses, liver toxicity at the doses of 250 and 500 mg / kg body weight; and disrupted rat lipid, protein and carbohydrate metabolism at the dose of 500 mg / kg body weight. All the registered toxic effects are reversible.

Moreover, the extract does not induce delayed toxic effects.

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