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## Original Research Article

# Effects of the ethanolic extract of *Piliostigma reticulatum* DC (Horscht) stem bark on biochemical parameters of albino rat wistar

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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. To verify its safety, the ethanolic extract of the stem bark is orally administered for 28 days in three groups of rats at doses of 250, 500 and 1000 mg / kg body weight. The control group received distilled water. The blood collected every week is used to assay biochemical parameters. At all doses, the ethanolic extract of the stem bark of *Piliostigma reticulatum* did not affect the serum values of transaminases, urea, total protein, triglycerides and bilirubin values compared to the control group. In return, it resulted in all dose levels, an increase in serum creatinine in the second week and an increase in serum cholesterol in a dose of 1000 mg / kg body weight at the first and fourth week. Our sample resulted in the fourth week, hypoglycemia in the group treated at 1000 mg / kg body weight. All these observed effects are reversible. Furthermore, it does not cause delayed toxicity.
**Keywords:** Biochemical parameters, ethanolic extract, *Piliostigma reticulatum*, rat, reversible, safety.

## INTRODUCTION

The use of medicinal plants for health care is a practice that is gaining popularity worldwide. With this in mind, WHO recommends the use of herbal medicine whose safety, efficacy and quality are guaranteed [25]. *Piliostigma reticulatum* is one of the medicinal plants of the ivorian rich flora [2], used for the treatment of several diseases such as colic, hemorrhoids, especially diarrhoea [31, 9]. *Piliostigma reticulatum* is a tree of 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 beneath. They are heavily lobed with rounded lobes or corner. The fruit is a woody pod, flat, hairless, sometimes twisted and cracked [5]. For the traditional treatment of diarrhoea, alcohol macerated of *Piliostigma reticulatum* stem bark is used in beverage for two or three days [9]. Pharmacological studies on this plant have shown that the leaves of *Piliostigma reticulatum* have antibacterial and antimicrobial properties [3]. The sedative and anticonvulsant properties of leaves were also highlighted [8]. With an estimated $LD_{50}$ more than 5000 mg / kg of body weight, total ethanolic extract of stem bark of *Piliostigma reticulatum* reduced diarrhoeic feces at doses of 250, 500 and 1000 mg / kg body weight [9]. These authors showed that this sample to 500 and 1000 mg / kg body weight also inhibits the gastro intestinal motility. Despite these multiple biological activities, safety-related studies have not been conducted. The aim of this study is to evaluate the subacute toxicity of total ethanolic extract of stem barks of *Piliostigma reticulatum* through its effects on some biochemical parameters.

## MATERIAL AND METHODS

### Plant material

*Piliostigma reticulatum* stems bark were collected in January 2015 in Kadjabo in the township of Dimbokro located about 240 km from Abidjan. The identification was confirmed by the National Centre for Floristic of Abidjan where a sample is deposited under number 18033.

### Animal

Rats of the species *Ratus norvegicus*, wistar strain aged 4 to 6 weeks, weighing between 97 and 108 g were used for the test. They come from our pet store. All animals were subjected to a temperature of 25 ± 2 ° C with alternating 12 hours of light and 12 hours dark. They were fed pellets of FACI® and were given tap water ad libitum in bottles without discontinuity.

### Preparation of total ethanolic extract of the stem bark of *Piliostigma reticulatum*

The stem bark of *Piliostigma reticulatum* are dried in the laboratory at a temperature of 25 ± 2 ° C for two weeks and pulverized using a mixer brand

RETSH type SM 100. Fifty grams of this powder are soaked in 1L of ethanol solution (96º) / water (80:20) for 24 hours with magnetic stirring. The extract is filtered through cotton wool and then on paper whattman's No. 1. The filtrate was evaporated under vacuum using a rotary evaporator type BUCCHI R 110 / NKE 6540/2. The extracts were introduced in an oven at 45ºC for 48 hours to obtain powders that constitute the total ethanolic extract of *Piliostigma reticulatum* stem bark [9].

**Study of subacute toxicity**

The study of subacute toxicity is conducted according to the OECD 407 guideline [24]. Sixty rats were divided into six groups comprising four tests groups and two control groups. Each group consisted of 10 rats with 5 males and 5 females. Three doses were selected based on the work of Dosso *et al*.[9]. Doses 250, 500 and 1000 mg / kg body weight (bw) are administered to groups respectively 2, 3 and 4. Group 1 was received distilled water. The animals are individually marked and dosed orally an equal volume of vehicle to 2 ml / 100 g bw. The groups 5 and 6 are satellite groups receiving respectively distilled water and the ethanolic extract of *Piliostigma reticulatum* at a dose of 1000 mg / kg bw. The latter two items were used to study the reversibility, persistence, or delayed occurrence of toxic effects for 14 days after stopping treatment.

**Blood samples**

The day of sampling, the animals are fasted from 10 p.m. to 7:00 in the morning without food but with water at will [24]. The morning they were anesthetized with ether and blood is collected by the technique of incising the tip of the tail which is previously disinfected with alcohol 96 ° [18]. Blood is collected into dry tubes. These samples are taken in all rats one day prior to the administration of the extract, and weekly in the first four groups. Fourteen days after cessation of treatment, blood in the satellite groups of rats is taken.

**Blood biochemical examinations**

The glycaemia is determined directly from whole blood using a glucometer brand Accu-Chek® (Roche Diagnostics) according to the glucose oxidase method [30]. The blood contained in each dry tube was centrifuged at 3000 revolutions / minute for 5 minutes and the serum obtained was used for the measurement of other biochemical parameters. Glutamate pyruvate transaminase (GPT) and oxaloacetates (GOT) are determined by the kinetic method [13], total cholesterol and urea by the enzyme method [4, 14, 29]. Creatinine [10], total protein and triglycerides [11] were determined by the colorimetric method. Bilirubin is measured by the diazo method [21].

**Statistic analysis**

Graph pad software version 5.01 was used for processing the data obtained. Values are presented as the averages followed by the standard error mean (SEM). The comparisons of means are made relative to the control, due to the repeated measures ANOVA with mixed model, followed by Bonferroni post hoc test for a 95% confidence interval. The differences are significant if the p value is less than 0.05 [15].

**RESULTS**

Prior to administration of the extract, the values obtained on day 0 for all the studied parameters of the different groups were nearly equal to those from the control group (Figure 1 to 3 and Table I to III). Similarly, glucose and cholesterol levels did not significantly change known to 250 and 500 mg / kg b.w. during the experiment. But, after four weeks of administration of the extract, blood glucose treated rats at the dose of 1000 mg / kg b.w. decreased significantly ($p < 0.05$) compared to the control group (Figure 1). Moreover, cholesterol of treated rats at the dose of 1000 mg / kg b.w. increased significantly ($p < 0.05$) to the first and fourth week (Figure 2). Throughout the period of administration of the ethanol extract of the stem bark of *Piliostigma reticulatum*, serum triglycerides in all treated groups did not undergo major changes compared to the control group. As to creatinine, a very significant increase in serum ($p < 0.01$) was observed at the second week at all doses studied (Figure 3). However, any disturbance in serum urea and total protein was observed in all treated groups compared to control group. Also, the level of GOT, GPT and bilirubin in the serum of treated groups were not significantly different when compared to the values of the control group (Table II).

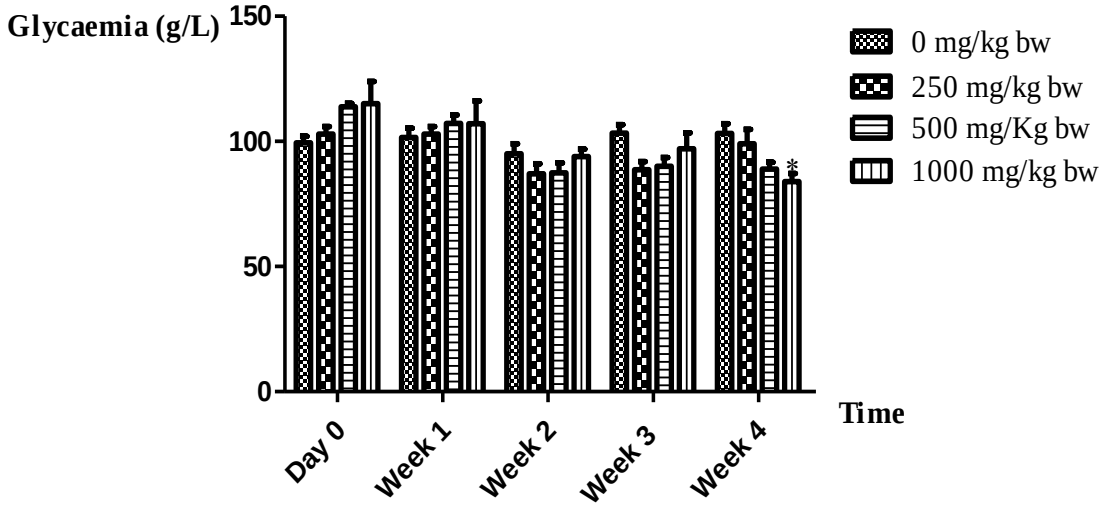


**Figure 1**: Effect of ethanolic extract of *Piliostigma reticulatum* stem bark on glycaemia, n=10, *: Statistically significant difference compared to the group 0 mg/kg bw ($p < 0.05$).

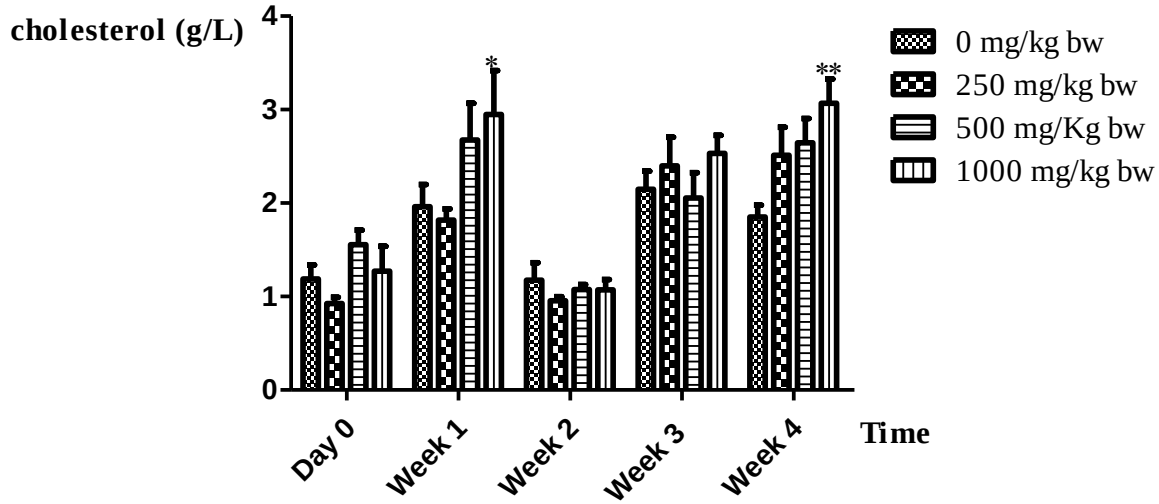


**Figure 2**: Effect of ethanolic extract of *Piliostigma reticulatum* stem bark on cholesterol, n=10, *: statistically significant difference compared to the group 0 mg/kg bw ($p < 0.05$),** statistically highly significant difference compared to the group 0 mg/kg bw ($p < 0.01$).

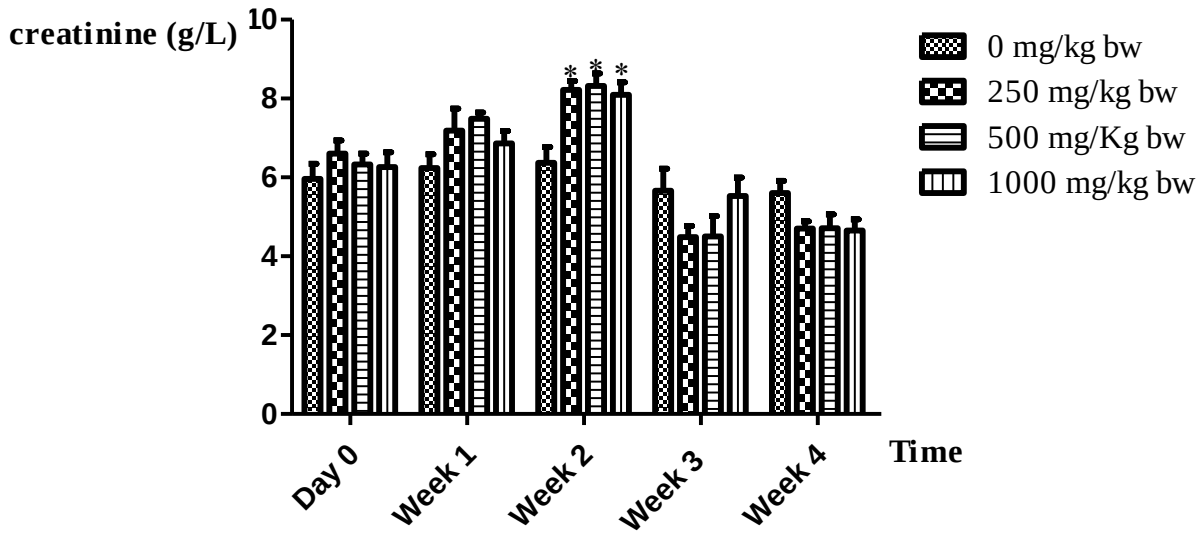


**Figure 3**: Effect of ethanolic extract of *Piliostigma reticulatum* stem bark on creatinine, n=10, *: Statistically significant difference compared to the group 0 mg/kg bw ($p < 0.05$).

**Table I: Effect of the ethanolic extract of *Piliostigma reticulatum* on biochemical parameters of the liver and kidneys.**

| | **Dose (mg/kg b.w.)** | **D 0** | **w 1** | **w 2** | **w 3** | **w4** |
|---|---|---|---|---|---|---|
| **Triglycerides (g/L)** | 0 | 0.8188±0.1387 | 0.8680±0.1346 | 0.7650±0.01688 | 0.6350±0.09459 | 0.6277±0.09765 |
| | 250 | 0.7446±0.09611 | 0.8000±0.09517 | 0.8050±0.05683 | 0.7020±0.07912 | 0.7015±0.09179 |
| | 500 | 0.8326±0.1193 | 0.9130±0.1491 | 0.6980±0.04742 | 0.6950±0.04362 | 0.6853±0.05266 |
| | 1000 | 0.7797±0.04363 | 0.8490±0.05630 | 0.7690±0.03860 | 0.5760±0.09432 | 0.5529±0.08076 |
| **Total proteins (g/L)** | 0 | 78.32±4.429 | 81.32±4.300 | 88.25±5.291 | 82.78±4.246 | 82.96±5.337 |
| | 250 | 86.26±6.178 | 88.18±6.131 | 81.59±9.537 | 89.82±5.726 | 87.06±4.917 |
| | 500 | 87.79±4.369 | 87.33±5.370 | 88.63±5.119 | 87.95±4.588 | 85.71±3.536 |
| | 1000 | 93.49±6.419 | 92.07±7.020 | 89.84±7.458 | 91.53±6.931 | 95.11±6.799 |
| **Urea (g/L)** | 0 | 2.589±0.1769 | 2.500±0.1905 | 2.664±0.2047 | 2.700±0.1606 | 2.858±0.1845 |
| | 250 | 2.713±0.1876 | 2.450±0.1364 | 2.938±0.1488 | 2.778±0.3403 | 3.116±0.3094 |
| | 500 | 2.682±0.1237 | 2.460±0.1300 | 2.910±0.2173 | 2.710±0.3455 | 3.294±0.3830 |
| | 1000 | 2.660±0.3159I | 2.700±0.2366 | 2.841±0.2480 | 2.800±0.3091 | 3.522±0.2643 |

n = 10 for each week, testing groups are compared to dose 0 mg / kg bw; D 0 = 1 day before feedings; w1, w2, w3, w4 respectively first
, 2nd, 3rd and 4th weeks; threshold of significance $\alpha = 0.05$.

**Table II Effect of the ethanol extract of *Piliostigma reticulatum* on biochemical markers of liver**

| | **Doses mg/kg b.w.** | **D 0** | **w 1** | **w 2** | **w 3** | **w4** |
|---|---|---|---|---|---|---|
| **GPT (UI/L)** | 0 | 9.998±0.5123 | 11.15±0.6165 | 12.74±0.4383 | 12.59±0.8479 | 14.35±0.8131 |
| | 250 | 9.111±1.505 | 11.34±1.515 | 12.29±0.4116 | 11.57±2.097 | 13.78±0.5872 |
| | 500 | 11.83±0.4488 | 13.33±0.6655 | 13.54±0.9157 | 15.00±1.057 | 15.09±0.9220 |
| | 1000 | 10.84±1.342 | 12.25±1.630 | 12.71±0.9421 | 13.73±1.985 | 14.34±1.307 |
| **GOT (UI/L)** | 0 | 31.56±1.023 | 30.34±1.505 | 31.52±1.533 | 30.74±1.621 | 30.07±1.425 |
| | 250 | 30.31±1.626 | 32.77±2.627 | 28.61±3.883 | 32.62±2.926 | 33.97±2.209 |
| | 500 | 25.23±1.873 | 31.89±4.019 | 33.26±1.668 | 36.38±3.157 | 37.17±2.634 |
| | 1000 | 31.68±2.204 | 33.83±3.753 | 32.52±1.184 | 33.98±2.974 | 38.48±2.060 |
| **Total Bilirubin (g/L)** | 0 | 3.086±0.4222 | 2.601±0.3283 | 2.585±0.3056 | 2.567±0.3657 | 2.876±0.3609 |
| | 250 | 4.283±0.6070 | 3.589±0.5340 | 3.649±0.5660 | 3.595±0.5681 | 3.958±0.5770 |
| | 500 | 3.013±0.2607 | 2.497±0.2500 | 2.556±0.2438 | 2.435±0.1959 | 2.797±0.2628 |
| | 1000 | 3.716±0.3756 | 3.027±0.2846 | 3.160±0.3003 | 2.931±0.2492 | 3.486±0.3732 |
| **Direct Bilirubin (g/L)** | 0 | 1.064±0.1337 | 0.8900±0.1167 | 0.9000±01137 | 0.8840±0.1194 | 0.9857±0.1271 |
| | 250 | 1.491±0.2227 | 1.248±0.1880 | 1.253±0.1815 | 1.240±0.1906 | 1.365±0.1932 |
| | 500 | 1.042±0.09929 | 0.8602±007203 | 0.8710±0.07131 | 0.8494±0.06661 | 0.9579±0.08702 |
| | 1000 | 1.275±0.1306 | 1.053±0.09904 | 1.066±0.09643 | 1.039±0.08988 | 1.178±0.1247 |

n = 10 for each week, testing groups are compared to dose 0 mg / kg bw; D 0 = 1 day before feedings; w 1, w 2, w 3, w 4 respectively 1rst, 2nd, 3rd and 4th weeks; threshold of significance $\alpha = 0.05$.

After discontinuation of the administration of the ethanolic extract of the stem bark of *Piliostigma reticulatum* , no significant change of the parameters of the treated rats were observed compared to the control group **(Table III)**. Slight variations were noted at the end of the experiment at a dose of 1000 mg / kg body weight all disappeared after discontinuation of treatment. There was a decrease in blood glucose, increased serum cholesterol and creatinine.

**Table III: Effects of ethanolic extract of *Piliostigma reticulatum* on biochemical parameters of satellite rats.**

| | **Dose mg/kg bw** | **D0** | **w6** |
|---|---|---|---|
| **Glycaemia (g/L)** | 0 | 104.8 ± 4.284 | 102.3 ± 3.865 |
| | 1000 | 108.3 ± 3.413 | 99.40 ± 14.43 |
| **Cholestérol (g/L)** | 0 | 1.466 ± 0.4440 | 1.539 ± 0.4962 |
| | 1000 | 1.244 ± 0.2325 | 1.121 ± 0.2185 |
| **Triglycerides (g/L)** | 0 | 0.6568 ± 0.07483 | 1.264 ± 0.1703 |
| | 1000 | 0.7210 ± 0.07223 | 2.071 ± 0.4238 |
| **Creatinine (g/L)** | 0 | 6.232 ± 0.2778 | 5.619 ± 0.3867 |
| | 1000 | 5.413 ± 0.2971 | 5.258 ± 0.2222 |
| **Urea (g/L)** | 0 | 2.633 ± 0.3073 | 2.900 ± 0.3966 |
| | 1000 | 2.561 ± 0.1776 | 3.307 ± 0.3046 |
| **Total Proteins (g/L)** | 0 | 87.64 ± 6.721 | 83.76 ± 5.300 |
| | 1000 | 81.15 ± 7.782 | 77.72 ± 7.429 |
| **TGP (UI/L)** | 0 | 11.45 ± 1.640 | 12.85 ± 1.829 |
| | 1000 | 12.74 ± 1.969 | 14.15 ± 2.108 |
| **TGO (UI/L)** | 0 | 31.29 ± 2.205 | 33.05 ± 3.105 |
| | 1000 | 29.50 ± 2.261 | 34.31 ± 4.162 |
| **Total Bilirubin (g/L)** | 0 | 2.293 ± 0.2856 | 2.598 ± 0.4072 |
| | 1000 | 2.747 ± 0.3500 | 3.134 ± 0.3411 |
| **Direct Bilirubin (g/L)** | 0 | 0.8201 ± 0.1237 | 0.9066 ± 0.1355 |
| | 1000 | 0.9815 ± 0.1321 | 1.063 ± 0.1193 |

n = 10 for each week, testing group is compared to dose 0 mg / kg bw; D 0 = 1 day before feedings, S 6 = 6th weeks; threshold of significance $\alpha = 0.05$

**DISCUSSION**

Prior to administration of the ethanolic extract of *Piliostigma reticulatum* stem bark, all rats used for the experiment were in the same physiological condition for the determination of biochemical parameters revealed identical values. During the experiment, serum creatinine increased in all treated rats at the second week. This result is similar to that of

other authors who have shown an increase in serum creatinine in rats treated with the aqueous extract of *Spondias mombin* stem bark at doses 500 and 1000 mg / kg bw [22]. Creatinine and urea are markers of renal function. Thus, an increase of their rate reflects renal dysfunction [20]. It therefore appears signs of nephrotoxicity after two weeks of treatment. However, it seems that this is transitory nephrotoxicity because she had gone to the third and fourth week of the experiment. Transaminases reflect liver function, particularly glutamate pyruvate transaminase [26]. An increase of their rate indicates liver damage [16]. During the period of administration of the extract, serum transaminases of the treated groups were unchanged implying that the ethanol extract of the stem bark of *Piliostigma reticulatum* did not affect liver function. Previous works in our own have led to different results [12]. It has been observed an increase in serum GPT following repeated administration of the aqueous extract of *Allium sativum* to rats at a dose of 4800 mg / kg bw. They GOT serum level also provides information on the condition of skeletal and cardiac muscles [27]. Therefore, our findings suggest that the ethanol extract of the stem bark of *Piliostigma reticulatum* does not result in myocardial or skeletal muscles injuries of rats. This result is similar to that achieved by Koné *et al*. in their work on the subacute toxicity of the aqueous extract of *Sacoglottis gabonensis* stem bark in rats [17]. Bilirubin comes from the conversion of the heme released from the erythrocytes [28]. It may be in free or conjugated form. High bilirubin levels are due either to an inability of the kidneys to remove excess bilirubin or to a failure of the liver to combine effectively to its excretion [6]. Serum bilirubin levels did not change during the study, which supports the hypothesis that the extract tested would have no effect on the liver. This result is not consistent with that obtained in other experiments [23]. These authors showed with the aqueous extract of *Cochlospermum planchonii* rhizomes at a dose of 50 mg / kg bw, decreased bilirubin during an administration period between 1 and 5 days, followed by an increase between 10 and 15 days. A drop in proteinemia indicate a malfunction of microtubule system of hepatocytes [28]. The ethanol extract of *Piliostigma reticulatum* stem bark did not change the total protein. Our extract would therefore have no effect on the metabolism of proteins, particularly the microtubule system of rat hepatocytes. Increased levels of triglycerides and cholesterol promote arteriosclerosis which in turn is the cause of the majority of myocardial infarction [19]. Regarding the lipid profile, our extract resulted in an increase of the cholesterol to the first and fourth weeks for the dose of 1000 mg / kg bw but was without effect on triglycerides. Its effect is therefore directed specifically on the metabolism of cholesterol. This fact differs from that observed in the work of another authors [7].They did not observed any variation in cholesterol levels with the aqueous extract of *Elaeocarpus grandiflorus*. In the fourth week, the decline in blood glucose of rats at a dose of 1000 mg / kg bw suggests that the extract disrupt glucose metabolism in the fourth week. Results different from ours were obtained by administering about 28 days the aqueous extract of the stem bark of *Sacoglottis gabonensis* in rats [17]. Indeed, in their study, at doses of 3.5, 35 and 350 mg / kg bw, blood sugar has not changed. After stopping treatment, no change was observed for all studied parameters. This assumes that the ethanolic extract of the stem bark of *Piliostigma reticulatum* does not cause delayed toxicity. Furthermore, the toxic effects found during the experiment are reversible.

## CONCLUSION

This study found that the ethanol extract of the stem bark of *Piliostigma reticulatum* administered orally over 28 days, caused renal toxicity and a transient disturbance of lipid and carbohydrate metabolism. This extract is yet well tolerated by the body. However, the use of the ethanolic extract of *Piliostigma reticulatum* stem bark over a long period must be done with great caution since at high doses, it may cause side effects in humans. Histopathological analysis would be required to provide additional informations to those obtained in this study.

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