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Research Article

Subchronic toxicity of 70 % hydro-ethanolic extract of *Terminalia superba* trunk bark Engl. and Diels (Combretaceae) in *Wistar* rats

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ABSTRACT

Terminalia superba is a plant used in traditional medicine for the treatment of gastric ulcer in Côte d'Ivoire. The aim of this work was to study the subchronic toxicity of a 70 % hydro-ethanolic extract of *T. superba* via the assessment of some anthropometric, biochemical and histological parameters in *Wistar* rats.Thus, except 20 rats of group A served as control group, which received orally distilled water at 10 mL / kg b.w., three groups of 20 rats each of either sex, received daily, by oral route, 70 % hydro ethanolic trunk bark extract of *T. superba* for ninety days respectively 250 (group B), 500 (group C) and 750 (group D) mg/kg b.w. Two satellite groups of 10 rats each were constituted in order to check the possible reversibility of the side effects or the occurrence of delayed effects, 30 days after stopping the treatment i.e the 120th day. The rats were weighed every week. Blood samples were taken by ocular puncture on days 0, 60 and 90 and collected in dry tubes from fasted rats previously anesthetized with ether in order to assess 70 % HEE extract impact on some biochemical parameter levels such as amino transaminase (AST and ALT), direct and total bilirubin, urea, creatinine, potassium, sodium, triglycerides, total, HDL-cholesterol, LDL-cholesterols and glycemia. At the end of the 90 or 120 days, rats were sacrificed and the liver, the kidneys and the heart were removed and weighed. The relative organ weights were determined and a histological study was carried out on these organs. The analysis of the results showed that the weight gain of all rats treated with 70 % HEE was significantly ($P < 0.05$) lower than control rats. 7The 70 % HEE did not interfere with the relative organ weights of the organs. This extract caused a significant decrease ($p < 0.05$) in total bilirubin, potassium, triglycerides levels at 750 mg/kg b.w. and induced a significant ($p < 0.05$) increase in creatinine and HDL-c levels at the same dose. The histological analysis revealed hepatic infiltration and steatosis, atrophied glomeruli in rats treated with 70 % HEE at 750 mg/kg b.w. were observed. HEE 70 % did not induce any damage to heart. However, abnormalities seen in liver and kidneys resolved after stopping the administration of HEE 70 %. In conclusion, HEE 70 % administered at 500 mg/kg b.w. was not toxic but at 750 mg / kg b.w. (high dose), it involved reversible body damages.

***Key words*:** *Terminalia superba*, 70 % hydro-ethanolic extract, anthropometric, biochemical parameters, histological study

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INTRODUCTION

Helicobacter pylori is one of the primary causes of gastric ulcer disease [1]. The elimination of this bacterium not only significantly permits to reduce the risk of recidivism but to treat this pathology efficiently [2]. Many drugs were used to treat this pathology in Allopathic medicine [1]. However, despite the diversity of the drugs resulting from this medicine, gastric ulcer has not yet been eradicated, hence the use of the medicinal plants by some populations to treat themselves [3]. The traditional use of the medicinal plants did not exclude the risks of intoxication which can prove to be fatal [4]. To prevent such risks, the Laboratory of Physiology, Pharmacology and Pharmacopoeia of Nangui Abrogoua University of Abidjan (Côte d'Ivoire) has initiated research work on *Terminalia superba* (Combretaceae), a plant used in the treatment of gastric ulcer in Côte d'Ivoire. The pharmacological study undertaken by aforesaid laboratory, revealed goods and interesting results in the effectiveness of this plant using aqueous and 70 % hydro-ethanolic extract (70 % HEE) in the treatment of ulcers especially at 500 mg/kg b.w. [5, 6]**.** Moreover, no scientific research work has been done concerning its effects in repeated administration for ninety days. Hence, the present study was undertaken to assess the effects of 70 % HEE at 250, 500 and 750 mg/kg of b.w. in repeated administration for 90 days on anthropometric, biochemical and histological parameters in *Wistar* rats.

MATERIAL AND METHODS

Plant material

Fresh trunk barks of *T. superba* were collected locally from the forest of Ebillassokro village in the East of Côte d'Ivoire. Taxonomic identification and authentication were established by botanist from the National Floristic Center of University of Felix Houphouët Boigny, Cocody- Abidjan, Côte dIvoire, voucher n°2456, *T. superba* Engl. and Diels in June 4, 1954; n°4207 in March 26, 1957; n°10477, February 26, 1969 and n°416 in April 03, 1974 of Côte d'Ivoire national herbarium.

Animals

Albino male and female *Wistar* rats of species *Rattus norvegicus* weighing between 90 and 116 g, approximately 6–8 weeks old were used. They were fed with pellets FACI® and water *ad libidum* in the Animal House of Physiology, Pharmacology and Pharmacopeia laboratory of the University of Nangui Abrogoua (Abidjan, Côte d'Ivoire). They were exposed to 12 hours dark/light cycle at room temperature (22–25 °C). The various experimental protocols were followed in accordance with the protocols for the protection of laboratory animals of the European Council of Legislation 2012/707 / EU [7]**.**

Chemical substances

Ether (VWR International-Geldenaakfebaan 464-B-3001, Leuven, Belgium) and ethanol (Sigma Chemical Company, Saint Louis, MO, USA) were used for this study.

Methods

Preparation of the 70 % hydro-ethanolic extract of *Terminalia superba*

The trunk bark was collected and dried under shade (22-25 ° C) for two weeks. Once dried, it was finely powdered using an electric grinder type RETSCH SM100 (Germany). The extraction process was implemented according to the method described by [8]. Fifty grams of this trunk bark powder of *T. superba* were macerated for 24 hours in one-liter ethanol-water (70:30 v/v) for 3 times until complete exhaustion. The mixtures were stirred magnetically for 72 hours, filtered (cotton and Whatman n°1) and concentrated under reduce pressure using a rotary evaporator (Büchi R110, type MKE 6540/2, Germany) at 45°C. The concentrated extracts were stored in dessicators (Mark Fruicell, France) at 45 °C for 2 days. A black brown powder (9,56 g) which represents the hydro-ethanolic extract 70 % of the trunk bark of *T. superba* (70 % HEE) was obtained.

Subchronic toxicity study

It was carried out according to OECD guidelines 408 [7, 9] which consists in daily administering, 70 % hydro-ethanolic extract of *T. superba* **(**70 % HEE**),** increasing doses to four groups of rats at one dose per groups for 90 days orally.

One hundred rats were randomly divided in four groups of twenty animals including three test groups and one control group. Each group included ten male and ten female rats. The four groups corresponding to group A, B, C and D, received orally distilled water at 10 ml/kg b.w. and 70 % HEE at 250, 500 and 750 mg/kg b.w. respectively. Ten additional rats per group, including five males and five females served as satellite groups were added in groups A and D in order to check the possible reversibility or side effects or the occurrence of delayed effects at least 30 days after the end of the treatment [7]. Before various treatment administration, the animals of each group were marked and weighed individually and weekly to readjust the administered extract doses.

Biochemical study

In order to assess the basic values of each parameter in each group, a blood sample was collected on day 0 before the administration of 70 % HEE in all the rats then on days 60 and 90. The animals were fasted for 8 hours. They were slightly

anesthetized with cotton wool soaked in Ether. Blood samples from each fasted rat were collected by ocular puncture in dried tubes according to the method described by [10]. They were centrifuged at 3000 rpm for 5 min and the serum was subjected to the analysis of some serum biochemical parameters such as AST, ALT, total and direct bilirubins, creatinine, urea, sodium, potassium, total, HDL-c, LDL-c and triglycerides using a semi-automatic analyzer (Robonik ®, India). Glycemia was immediately carried out after blood withdrawal using a glucometer (Accu-check®, France).

Histological study

At the end of the ninety days, all the rats were euthanized with ether and were sacrificed except those from the satellite groups which underwent the same treatment thirty days after stopping treatment. The rats were dissected and the livers, kidneys and hearts were removed, weighed then subjected to macroscopic examinations. They were fixed in 10 % formalin for anatomopathological examinations by using the paraffin inclusion method described by [11]. Each removed organ was weighed in order to determine its relative organ weight [12].

Statistical analyzes

Data were performed using GraphPad Prism 5.01 software (San Diego, California, USA) and presented as mean ± standard error on mean (M ± SEM). Comparisons between treated groups and controls were made using Student's t test and one-way analysis of variance (ANOVA). Bonferroni test was used as post-hoc test. Values were considered statistically significant when $P < 0.05$.

RESULTS

1- Effect of 70 % hydro-ethanolic extract of *Terminalia superba* trunk bark on ponderal growth in rats

Before oral administration of the 70 % hydro-ethanolic extract of *Terminalia superba* trunk bark (70 % HEE) to the treated groups B, C and D, no significant variation ($p > 0.05$) in the initial weight of the rats was recorded compared to the control group A (Figure 1). The rats treated with HEE 70 % experienced a significant ($p < 0.05$) weak increase in weight compared to the control group A (Figure 1). Oral administration of 70 % HEE to the rats of the treated groups B, C and D for 90 days induced a significant ($p < 0.05$) lower weight gain compared to the control group A (figure 1).

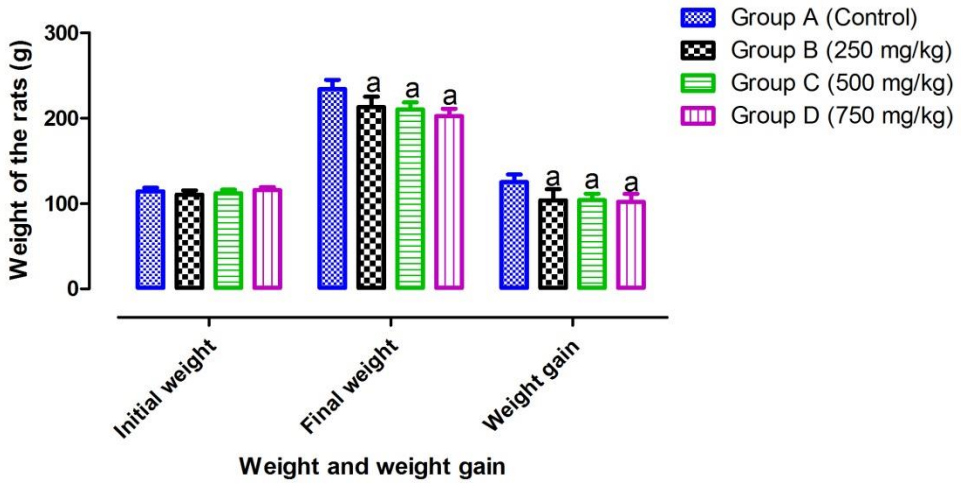

Figure 1: Effect of 70 % hydro-ethanolic extract of *Terminalia superba* trunk bark on weight growth and weight gain in rats for ninety days

n = 20 rats in each group. Comparisons were done between the control group A (distilled water group) and the treated groups B (70 % HEE, 250 mg/kg), C (70 % HEE, 500 mg/kg) and D (70 % HEE, 750 mg/kg); $^{a}P < 0.05$: significant difference recorded compared to the control group A.

2-Effect of 70 % hydro-ethanolic extract of *Terminalia superba* on organ weight and their relative organ weight.

As shown in Table 1, when rats were orally administered 70 % HEE at 250 (group B), 500 (group C) and 750 (group D) mg / kg b.w., no significant variation ($p > 0.05$) was recorded in the liver, kidneys and heart weights compared to their respective controls as well as that of their relative organ weights.

Table 1: Organ weight and relative organ weights after different treatment in rats

Organs	Groups	HEE 70% (mg/kg b.w.)	Organ weigt (g)	Relative organ weights (%)
Liver	**A**	**0**	5.150±0.139	32.57±1.661
	B	250	5.060±0.186	29.48±3.500
	C	500	4.97±0.193	30.28±1.90
	D	750	4.930±2.49	28.85±1.300
Kidneys	**A**	**0**	0.950±0.038	5.990±0.333
	B	250	0.986±0.041	6.378±0.313
	C	500	0.811±0.041	4.981±0.242
	D	750	1.060±0.031	6.939±0.231
Heart	**A**	**0**	0.642±0.015	4.054±0.181
	B	250	0.590±0.034	3.778±0.155
	C	500	0.452±0.034	2.771±0.187
	D	750	0.597±0.029	3.91±0.176

Comparisons were done between the control group A (distilled water group) and the treated groups B (70 % HEE, 250 mg/kg), C (70 % HEE, 500 mg/kg) and D (70 % HEE, 750 mg/kg). no significant difference between treated and control groups was observed at $p<0.05$. n = 20 rats in each group.

3- Biochemical study

3-1-Effect of 70 % hydro-ethanolic extract of *Terminalia superba* trunk bark on liver biomarkers in rats

At day 0, no significant variation ($p> 0.05$) in AST and ALT levels in the treated groups B (70 % HEE, 250 mg / kg), C (70 % HEE, 500 mg / kg) and D (70 % HEE, 750 mg / kg) was recorded compared to the control group A (Table 2). Oral administration of 70 % HEE did not induce a significant change ($p> 0.05$) in AST and ALT levels between the treated groups and the control group A on days 60 and 90 (table 2). As for total bilirubin level, very ($p <0.01$) and highly ($p <0.001$) significant decreases were observed respectively on the sixtieth and ninetieth day of the treatment when 70 % HEE was administered to the rats at 750 mg / kg b.w. compared to the control group A (Figure 2). In contrast, oral administration of 70 % HEE did not induce a significant change ($P> 0.05$) in direct bilirubin level of the treated groups compared to the control group A (Table 2).

Table 2: Evolution of liver serum biochemical parameters after different treatments in rats

Biochemical parameters	Groups	Day 0	Day 60	Day 90
AST	Group A	183±12	167.5±9.56	175.7±10.55
	Group B	192.7±9.10	159.6±10.73	186.3±6.50
	Group C	197.6±7.82	138.7±7.97	148.8±6.14
	Group D	187.7±9.43	177.5±8.37	158.6±6.46
ALT	Group A	95.49±6.83	90.59±8.46	94.97±2.26
	Group B	91.03±7.47	90.25±9.18	98.6±3.14
	Group C	97.2±8.67	93.02±5.27	87.33±6.07
	Group D	95.72±7.8	85.21±10.03	90.93±9.04
Direct bilirubin	Group A	0.379±0.027	0.415±0.016	0.413±0.036
	Group B	0.359±0.035	0.438±0.021	0.403±0.029
	Group C	0.388±0.025	0.419±0.020	0.363±0.014
	Group D	0.398±0.047	0.338±0.030	0.330±0.013

Comparisons were done between the control group A (distilled water group) and the treated groups B (70 % HEE, 250 mg/kg), C (70 % HEE, 500 mg/kg) and D (70 % HEE, 750 mg/kg); no significant difference was recorded compared to the control group A at $p < 0.05$. n=20 rats in each group.

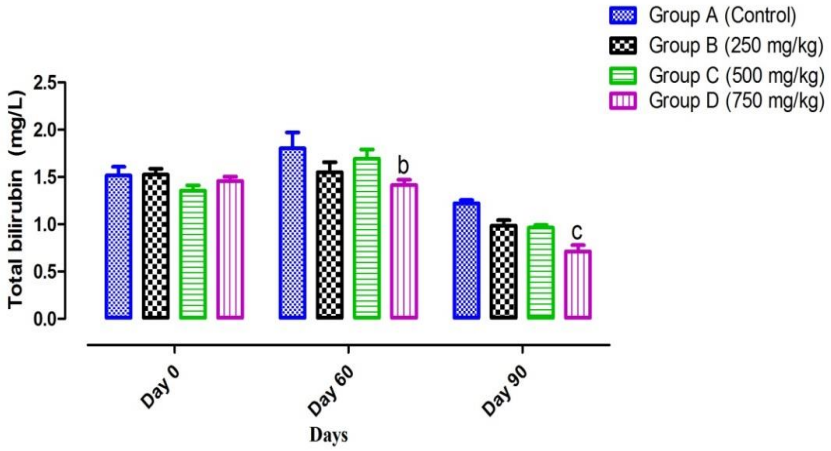

Figure 2: Effect of 70 % hydro-ethanolic extract of *Terminalia superba* trunk bark on total bilirubin in rats

Comparisons were done between the control group A (distilled water group) and the treated groups B (70 % HEE, 250 mg/kg), C (70 % HEE, 500 mg/kg) and D (70 % HEE, 750 mg/kg) at different period of treatment. $^{b}P<0.01$; $^{c}P<0.001$: significant difference compared to control group A, n = 20 rats per group.

3-2-Effect of % hydro-ethanolic extract of *Terminalia superba* trunk bark on kidney biomarkers in rats

On day 0, the levels of creatinine, urea, potassium and sodium were not significantly ($p > 0.05$) changed in rats. Oppositely, when 70 % HEE was administered to rats, the creatinine level of the treated groups B, C and D was not significant ($p > 0.05$) on the sixtieth day compared to the control group A. However, in the ninetieth, 70 % HEE induced significant ($p < 0.05$) and highly significant ($p < 0.001$) increases in rats treated with 70 % HEE at 500 and 750 mg / kg b.w compared to the control group A (Figure 3-A). As for the serum urea level, a very significant decrease ($p < 0.01$) at 500 mg / kg bw (70 % HEE) compared to the control group A on the 60th day was recorded (Figure 3-B). Likewise, oral administration of 70 % HEE did not involve significant modification ($P>0.05$) in sodium level from the 60th to the 90th day compared to the control group A (Table 2). Moreover, potassium level on the ninetieth day underwent a highly significant ($P < 0.001$) decrease in the treated groups (B, C and D) compared to the control group A (Figure 3-C).

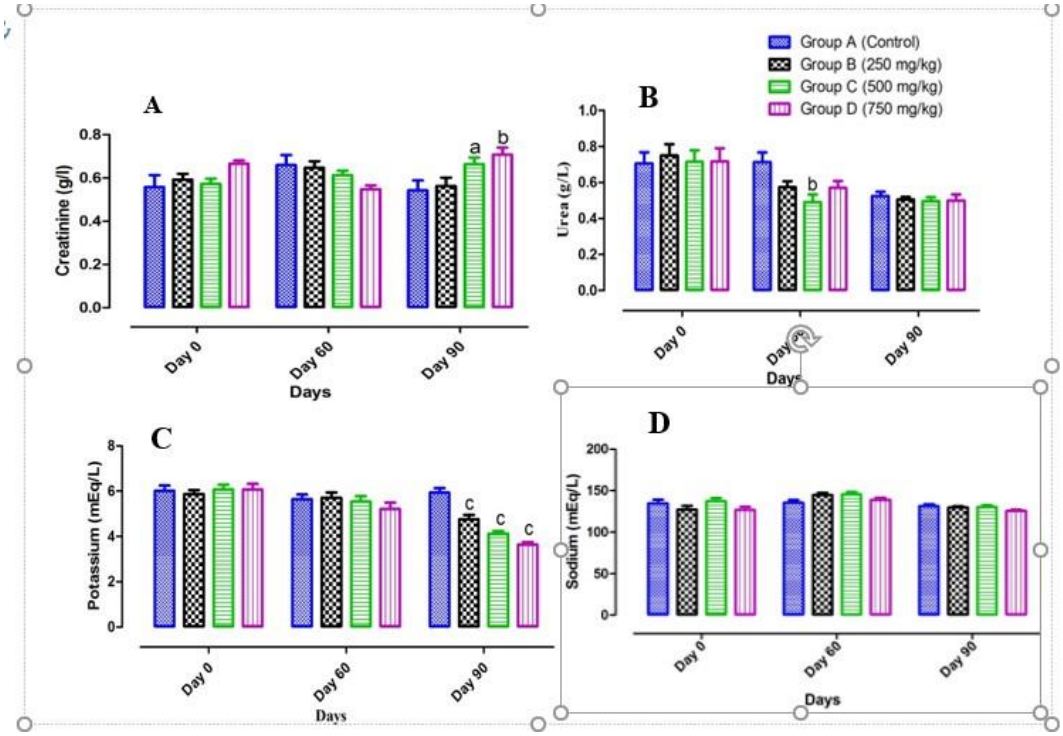

Figure 3: Effect of 70 % hydro-ethanolic extract of Terminalia superba trunk bark on kidney bio-markers in rat

A: Creatinine; B: Urea; C: Potassium; D: Sodium

Comparisons were done between the control group A (distilled water group) and the treated groups B (70 % HEE, 250 mg/kg), C (70 % HEE, 500 mg/kg) and D (70 % HEE, 750 mg/kg) at different period of treatment. ap<0.05; bp<0.01; cp<0.001: significant difference compared to the control group A, n=20 rats in each group

3-3-Effect of 70 % hydro-ethanolic extract of *Terminalia superba* trunk bark on lipid and glycemia profiles in rats

Generally, triglycerides, total, LDL, HDL cholesterols and glycemia levels of groups B (250 mg/kg), C (500 mg/kg) and D (750 mg/kg) before any treatment (day 0) with 70 % HEE did not significantly change (P>0.05) compared to the control group A (figures 4-A and B; Table 3). However, when 70 % HEE is administered to rats orally, a significant (p<0.05) reduction in triglyceride levels in the ninetieth day of the treatment at 750 mg/kg b.w. compared to the control group A was recorded (Figure 4-A). As for total and LDL cholesterol levels no significant (P>0.05) difference was observed between the groups treated with 70 % HEE and the control group A from the sixtieth to the ninetieth day (Table 3). Regarding the level of the serum HDL cholesterol, it lowered in a very significant manner (P<0.01) at 750 mg/kg b.w (group D) compared to the control group A on the sixtieth day (figure 4-B).On the other hand, on the ninetieth day, a highly significant increase (P<0.001) in HDL cholesterol level was recorded at 500 and 750 mg/kg b.w. with 70 % HEE compared to the control group A (Figure 4-B). Regarding glycemia level, oral administration of 70 % HEE did not involve significant change (P>0.05) in treated rats at all doses compared to the control group A in the sixtieth and ninetieth day (Table 3).

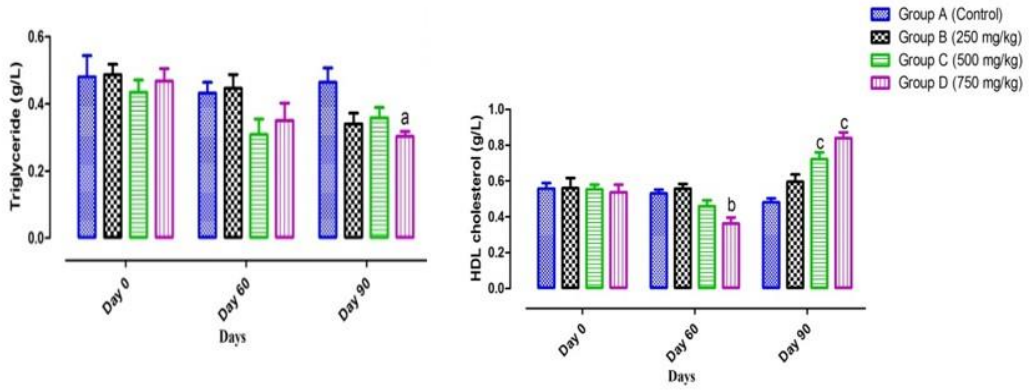

Figure 4: Effect of 70 % hydro ethanolic extract of *Terminalia superba* trunk bark on rat triglycerides and HDL cholesterol profiles

A: Triglycerides; B: HDL Cholesterol

[a]P<0.05; [b]P<0.01; [c]p<0.001: significant difference from control group A, n=20 rats in each group

Table 3: Evolution of lipidic and glycemic profiles after 90 days treatment in rats.

lipidic and glycemic parameters	HEE 70 % (mg/kg b. w.)	Day 0	Day 60	Day 90
Total cholestérol	0	0.604±0.027	0.470±0.034	0.687±0.028
	250	0.653±0.024	0.500±0.051	0.566±0.049
	500	0.654±0.028	0.546±0.062	0.663±0.065
	750	0.657±0.028	0.533±0.036	0.583±0.021
LDL cholestérol	0	0.153±0.022	0.151±0.019	0.151±0.020
	250	0.157±0.030	0.151±0.027	0.155±0.017
	500	0.166±0.034	0.155±0.027	0.156±0.026
	750	0.152±0.025	0.156±0.029	0.154±0.017
Glycemia	0	1.152±0.030	1.336±0.037	1.142±0.064
	250	1.068±0.029	1.345±0.061	1.144±0.077
	500	1.081±0.035	1.403±0.050	1.006±0.058
	750	1.144±0.033	1.329±0.086	1.143±0.057

Comparisons were done between control group A (70 % HEE, 0 mg/kg b.w.) and treated groups B (70 % HEE, 250 mg/kg b.w), C (70 % HEE, 500 mg/kg b.w) and D (70 % HEE, 750 mg/kg b.w.); no significant difference recorded compared to group A at $p < 0.05$. n=20 rats in each group.

4-Effect of the hydro-ethanolic extract 70 % of the trunk bark of *Terminalia superba* on rats' organs

Administration of 70 % HEE by oral route to rats produced normal livers in the control groups (Figure 5-A). In contrast, the livers of the rats treated with 70 % HEE showed steatosis at 750 mg / kg b.w. and infiltration at 500 and 750 mg / kg b.w. (Figure 5-B and 5-C). Histological sections of the control rats showed a normal occurrence in kidneys (Figure 5-D). On the other hand, atrophied glomeruli were observed at 750 mg / kg b.w. (Figure 5-E). Regarding cardiac tissue, no lesions were caused by oral administration of 70 % HEE for 90 days in the rats treated at 250 (group B), 500 (group C) and 750 (group D) mg / kg (Figure 6). Histological sections of rats' cardiac tissue showed normal heart tissue at 100 % and 0 % of cardiac lesions (Table 4).

Table 4: Toxicty effect on histological sections of cardiac tissue in rats

Organ	Groups	Normal hearts	Cardiac lesions
Heart	Group A	100 %	0 %
	Group B	100 %	0 %
	Group C	100 %	0 %
	Group D	100 %	0 %

Comparisons were done between the control group A (70 % HEE, 0 mg/kg b.w.) and the treated groups B (70 % HEE, 250 mg/kg b.w), C (70 %HEE, 500 mg/kg b.w) and D (70 % HEE, 750 mg/kg b.w.); no significant difference recorded compared to group A at $P < 0.05$. n=20 rats in each group.

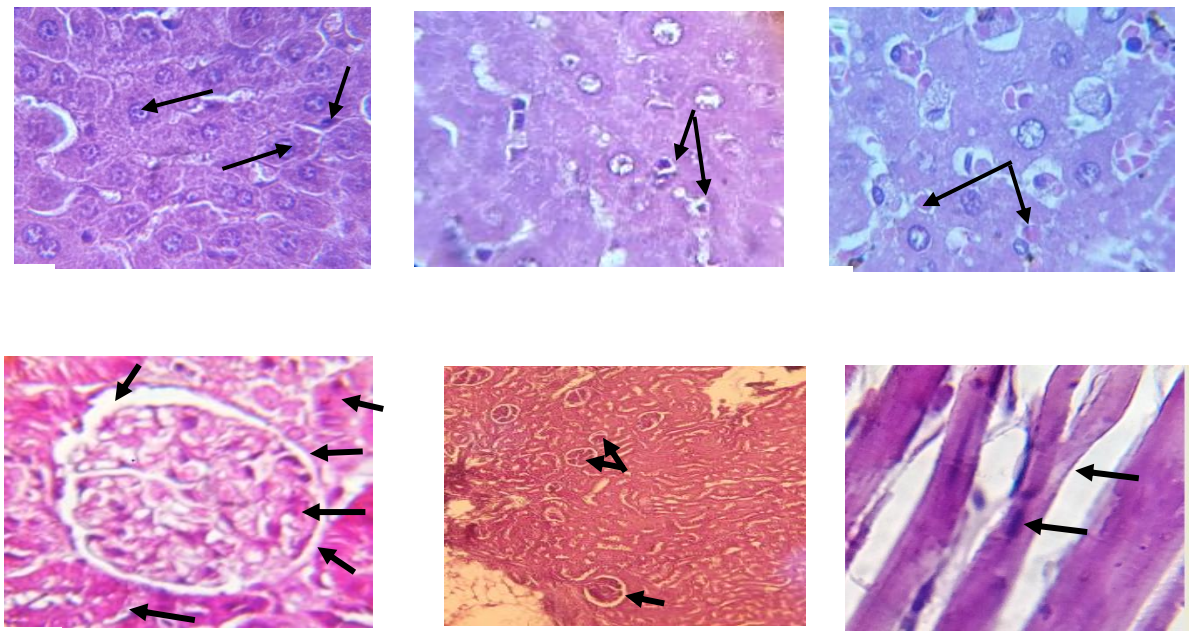

Figure 5: Histological sections of liver, kidney and heart in the control and groups treated with 70 % hydro-ethanolic extract of Terminalia superba trunk bark in rats

A: normal aspect of liver in the control group (x100); B: Steatosis in the group treated at 750 mg/kg b.w. (x100); C: Infiltration in groups treated with 70 % HEE at 500 and 750 mg/kg b.w. (x100); D: normal aspect of kidney in the control group (x100); E: Atrophied normal-sized glomerulus in the group treated with 70 % HEE at 750 mg/kg b.w. (x40); F: normal aspect of cardiac tissue in the control and treated groups (x100) colouring: eosin-hematoxylin, n : nucleus ; k : Küpffer's cell ; h : hepatocyte ; lv : lipidic vacuole ; bc : blood cells; fr: filtration room; pct: proximal convoluted tube; g: glomerulus; p: podocyte; Bc: Bowmann's capsule; dct: distal convoluted tube; ag: Atrophied normal-sized glomerulus; ng: normal-sized glomerulus; cc: cardiac cell.

5-Reversible and delayed effects of the hydro-ethanolic extract 70% of the trunk bark of

The repeated administration of 70 % HEE to rats, induced a significant (P<0.05) decrease in triglyceride levels in group D' compared to the control group A' on day 90. These significant variations, disappeared 30 days after the end of the treatment (Table 5). On the other hand, a significant (P<0.001) increase in creatinine and HDL cholesterol levels at 750 mg / kg bw compared to the control group A' on the ninetieth day was observed. These significant changes disappeared 30 days after the end of the treatment (Table 5). As for AST, ALT, direct bilirubin, urea, sodium, total, LDL cholesterols and glycemia levels, no modification was observed during 90th day of treatment. This absence of disruption, persisted 30 days after the end of the treatment (Table 5). Histologically, observation of liver sections revealed steatosis and infiltration in the rats treated with 70 % HEE at 500 and 750 mg / kg bw but after stopping treatment 30 days later, these abnormalities disappeared (Figure 6-A and 6-B). Regarding kidney sections, atrophied glomeruli were observed and 30 days after stopping treatment, no atrophied glomeruli were observed (Figure 6-C and 6-D).

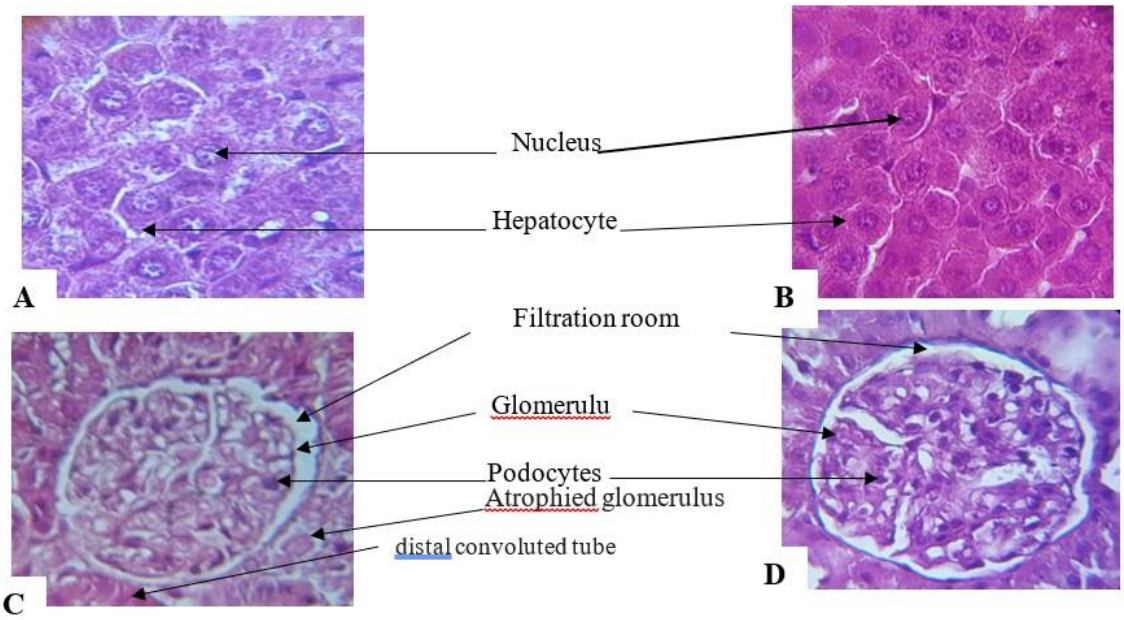

Figure 6: Histological sections of the liver and kidney at the end the treatment in rats

A: normal aspect of the liver in the control group A'**; B:** normal aspect of the liver in group D' treated with 70 % HEE at 750 mg/kg b.w; **C**: normal aspect of the kidney in the control group A'; **D**:normal aspect of the kidney in group D' treated with 70 % HEE at 750 mg/kg b.w; (x100); colouring: eosin-hematoxylin.

Table 5: Reversible effect on some biochemical parameters in rat

Parameters	Groups	Day 90	Day 120
AST (U/L)	A'	175.7±10.55	262.7±24.97
	D'	158.6±6.464	270.3±11.81
ALT (U/L)	A'	94.97±2.265	37.68±6.618
	D'	90.93±9.044	45.82±5.208
Total bilirubin (mg/l)	A'	1.220±0.036	0.447±0.044
	D'	0.712±0.066[c]	0.476±0.049
Direct bilirubin (mg/l)	A'	0.413±0.036	0.447±0.035
	D'	0.330±0.013	0.549±0.048
Creatinine (g/l)	A'	0.542±0.045	0.741±0.080
	D'	0.707±0.032[b]	0.827±0.053
Urea (g/l)	A'	0.524±0.023	0.517±0.022
	D'	0.500±0.033	0.408±0.027
Sodium (mEq/l)	A'	131.3±2.389	136.5±5.847
	D'	125.6±1.639	133.3±10.66
Potassium (mEq/l)	A'	5.937±0.197	3.647±0.036
	D'	3.639±0.112[c]	3.528±0.091
Triglycerides (g/l)	A'	0.463±0.042	1.936±0.202
	D'	0.302±0.015[a]	1.922±0.248
Total cholestérol (g/l)	A'	0.687±0.028	1.083±0.076
	D'	0.583±0.021	1.084±0.046
LDL cholesterol (g/l)	A'	0.151±0.020	0.379±0.066
	D'	0.154±0.017	0.187±0.058
HDL cholesterol (g/l)	A'	0.482±0.020	0.459±0.036
	D'	0.840±0.031[c]	0.692±0.070
Glycmia (g/l)	A'	1.142±0.064	0.422±0.019
	D'	1.143±0.057	0.422±0.033

Comparisons were done between the control group A' (distilled water group) and the treated group D' (70 % HEE, 750 mg/kg b.w). [a]p<0.05; [b]p<0.01; [c]p<0.001: significant difference compared to the control group A' (distilled water group). n = 10 animals in each group.

DISCUSSION

Oral administration of 70% hydro-ethanolic extract of of *Terminalia superba* trunk bark (70 % HEE) to rats for 90 days at all doses, induced lower weight growth and weight gain in the treated group than the control group. Increase or decrease in body weight has been used as an indicator of drugs and chemicals side effects [13]**.** Poor body weight gain in rats treated with 70 % HEE in this study may be the result of the appetite decreased and therefore the calorie intake decreased of the animals, as was shown for certain plants and their constituents, such as aristocholic acid isolated from many medicinal plants [14], and scabies from *Verbesina encelioides* [15]. According to [16], the mechanism of the plant side effects such as anorexia and weight loss was linked to an inhibiting effect of the gastric acid secretion. Low weight gain in the rats treated with 70 % HEE corroborated that of [17] who observed that the aqueous extract of the seeds of *Calycotome villosa* administered to rats for 90 days at 150, 300 and 600 mg / kg induced a slight weight gain but also a weight loss compared to the initial weight. The 70 % HEE was said to slow the weight growth of the rats.

Regarding the weight and the relative organ weights, 70 %HEE, did not cause any significant variation. These results were similar to those of [18], who reported that the aqueous extract of *Hermannia incana* leaves administered to rats for 90 days at 200; 400 and 600 mg / kg b.w. did not affect the relative organ weights. In addition, the relative organ weight is an index that provided information on swelling, atrophy or hypertrophy of the organ [19]. The absence of a significant difference between the relative organ weights (liver, kidney and heart) of the control rats and the treated one, suggested that 70 % HEE would have no effect on these organs. However, it is known

that an increase in relative organ weights would indicate hypertrophy or inflammation of the organ while a reduction in this could be atrophy [19]. The 70 % HEE does not induce any of the above changes.

Biochemically, the analysis of transaminases (AST and ALT) and bilirubins was used to evaluate liver functioning. The transaminases were released into the blood circulation in event of damaged cells. They are considered to be good indicators of hepatic cytolysis [20,21]. Their high level was attributable to the metabolic and / or toxic effects of drugs while their reduction was evidence of the liver proper functioning [22]. The significant decrease in these enzymes in treated animals would mean that the liver cells are not damaged by 70 % HEE. The decrease in the serum level of transaminases would be attributable to the presence of flavonoids in 70 % HEE 70 %. Flavonoids are free radical scavengers which react with them and protect hepatocytes [23]. The hepatoprotective effect of this extract against liver injuries could be attributable to Flavonoids in 70 % HEE [5]. These results are similar to those of [24] who have shown that administration of *Garcinia huillensis* extract at 4.5; 45; 450 and 1500 mg / kg to guinea pigs for 28 days, does not affect the level of transaminases. The results observed on day 90 during this study are similar to those of [25] who showed that the administration of ethyl acetate extract of *Holarrhena floribunda* leaves to rats at 1000 mg / kg b.w. for 90 days did not change the level of transaminases.

The majority of xenobiotics that enter the blood are excreted out of the body by kidneys [26]. Some of these xenobiotics could damage kidneys functioning. This renal damage is often manifested by the change in the value of certain biochemical markers [27]. Thus, the impact of 70 % HEE on kidney function was also evaluated during the subchronic toxicity study. Repeated administration of 70 % HEE induced a significant increase in serum creatinine at 500 and 750 mg / kg b.w after 90 days. On the other hand, in urea level, no significant variation was recorded until the 90th day. These results are contrary to those of [28] who observed that administration of the ethanolic extract of *Gongronema latifolium* leaves to rats at 150 and 300 mg / kg b.w. for 60 days, did not modify serum creatinine. According to [27], during medication, an increase in creatinine and urea levels was due to a change in the functional capacity of the renal tubules. In addition, [29] has showed that serum creatinine, exclusively linked to glomerular filtration, was a more accurate indicator of the assessment of renal function. These high levels in this study suggested that 70 % HEE has a toxic effect on renal function.

Lipid balance was carried out to assess the risk of the occurrence of cardiovascular diseases. It was essentially based on the determination of LDL-cholesterol, HDL-cholesterol and triglycerides, three parameters which are associated with the risk of the occurrence of cardiovascular diseases [30]. In this study, 70% HEE caused a decrease in triglyceride levels while HDL cholesterol increased significantly. However, an increase in total cholesterol and triglycerides levels was a risk factor for cardiovascular disease, while a decrease in serum triglyceride level and an increase in HDL cholesterolemia constitute a factor in their prevention [31]. These results differ from those of [32] who observed that the administration of ethyl acetate extract of *Holarrhena floribunda* leaves for 90 days at 1000 mg / kg b.w to rats did not affect the serum level of lipids. 70 % HEE administered to rats for 90 days did not induce a risk of cardiovascular disease. Better, it would have a protective effect.

The absence of modification in glycemia induced by oral administration of 70 % HEE differs from those obtained by [33] who recorded an increase in glycemia when the methanolic extract of *Harungana madagascariensis* trunk bark was administered to rats at a 400 mg / kg b.w. This result suggested that 70 % HEE did not affect insulin action over a long administration period. No change in glycemia was recorded. When the treatment was stopped, all the disturbances observed, disappeared and the values of all these biochemical parameters were normalized. The effect of the extract would therefore be transient and would be eliminated by the body.

It should therefore be remembered that the organ weights and their relative organ weights are indicators of the pathological or physiological state of the animal that a microscopic observation can highlight. It is on this basis that histological studies have been undertaken in this work. Thus, microscopic observation of the liver revealed steatosis and infiltration into liver cells at 750 mg / kg b.w. Infiltration is a lesion that responds to damage to an organ [34]. The 70 % HEE which resulted in hepatic cell infiltration was believed to induced liver damage at 750 mg / kg b.w. This assault could be the stress of the liver tissue on long term exposure. In addition, according to [35], steatosis was caused by poor oxidation of fatty acids or by toxin or drug actions. The 70 % HEE may interfere with the oxidation of fatty acids and cause fatty liver disease. These results are similar to [36] who showed that the administration of the methanolic extract of *Psidium guajava* trunk bark to rats at a 1000 mg / kg bw induced hepatic inflammation.

In kidneys, results indicated glomeruli atrophy at 750 mg / kg b.w. The 70% HEE may reduce kidney activity. The atrophy observed in this study could be due to the long exposure and to the high dose of 70 % HEE (750 mg / kg bw) which exceeded the therapeutic dose (500 mg / kg b.w). Indeed, [37] asserted that the secondary metabolites responsible for the therapeutic activity of medicinal plants are also the cause of toxicity when the dosage is high. These results corroborate those of [38] who showed that the aqueous extract of the trunk bark of *Spondias monbin* at 1000 mg / kg affected kidney tissue. Regarding heart, histological cross-sectional observations revealed no lesions. These results are similar to those of [39] who found that administration of the aqueous extract of *Khaya senegalensis*, *Mitragyna stipulosa* and *Kigelia africana* trunk bark to rats did not induce cardiac injury. On the other hand, these results are contrary to those of [40] who reported that the administration of the aqueous extract of *Morinda Lucida* leaves induced hemorrhage and swelling disorders of the heart muscle in rats that received 1626.5 mg / kg b.w The70 % HEE would not induce toxicity in heart at studied doses. In addition, all the abnormalities observed as well on liver and kidney levels disappeared after stopping treatment. These harmful effects are therefore reversible. Moreover, no anomalies appeared late.

CONCLUSION

This present work showed that 70 % HEE did not induced side effects on the organ weights and relative organ weights, lipidic metabolism, glycemia and heart tissue. However, this extract caused weak ponderal growth which induced a weak weight profit, a functioning disturbance of kidneys, hepatic steatosis and atrophied glomeruli especially beyond the therapeutic dose (500 mg/kg b.w.).These modifications observed are reversible and disappear after stopping treatment without letting appear delayed toxic effects.

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Ethical approval

The experiments were conducted in accordance with the protocols for the protection of laboratory animals of the European Council of Legislation 2012/707 / as reported in the Methodology section and all authors hereby declare that "Principles of laboratory animal care" were followed, as well as specific national laws where applicable.

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