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SUBACUTE TOXICITY STUDY OF THE AQUEOUS LEAVES EXTRACT OF *MACARANGA BARTERI* ON BIOCHEMICAL AND HISTOLOGICAL PARAMETERS IN RATS

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ABSTRACT
Macaranga barteri is commonly used in the treatment of many illnesses such as gastric ulcer. In order to verify its safety, a study was implemented with an aqueous extract of *M. barteri* leaves (AEMb) in rats. Seventy rats were distributed in seven groups of ten rats each of both sexes. The control group received orally 1 ml/100 g distilled water for 28 days while test groups 2, 3, 4 and 5 were repeatedly administered with AEMb *per os* at the doses of 125, 250, 500 and 1000 mg/kg b.w. respectively. Groups 6 and 7 (satellites groups) were daily gavaged with distilled water (1 ml/100 g) and AEMb (1000 mg/kg b.w.) for 28 days. At the end of the experiment, the rats blood samples were collected for serum biochemical parameters determination. In addition, samples of liver, heart and kidney were analyzed in order to evaluate possible side effects of the extract. The results showed that the doses of AEMb induced no significant variation of thes parameters. However, significant increases in serum chlorine and urea were observed in the rats treated with 1000 mg/kg b.w of AEMb. The histological analyses of the organs did not reveal any abnormalities. Moreover, the extract did not cause any delayed toxicity. The toxic effects previously observed in kidney disappeared two weeks after the end of the treatment. These results indicated that AEMb is safe. However, the administation of AEMb at 1000 mg/kg b.w. could impair the renal function in rats with reversible effects.

KEYWORDS: biochemical parameters, histology, *Macaranga barteri*, rat, subacute toxicity.

1- INTRODUCTION
Medicinal plants are valuable resources for people in Africa.[1] 80% of African population use plants to provide primary health care.[2] The accessibility of medicinal plants makes their use anarchic. Meanwhile, the ingestion of some plants can cause side effects and lead to death.[3] Indeed, cases of intoxication related to the use of medicinal plants have been reported. For instance, the extract of *Stephania tetrandra* and *Magnolia officinalis* used for weight loss remedies have been reported to cause nephrotoxicity.[4] The use of *Momordica charantia* for the treatment of amenorrhea has led to cases of fainting, vomiting and hyperthermia.[5] The WHO developed guidelines on medicinal plants in order to verify their efficacy and safety. Therefore, this study on *Macaranga barteri*, a plant widely used for the treatment of many conditions.[6,7] was initiated. Previous works have shown that *M. barteri* extracts have antioxidant,[8] anti-inflammatory[9], antimicrobial, anti fungal, [10,11] and anti-ulcer effects.[12] Moreover, Ehilé et al.[12] reported that the extract has a lethal dose 50% higher than 5000 mg / kg b.w. by using OECD Guideline 420.[13] In a subacute oral toxicity study, we showed that the aqueous extract had few side effects on hematolological parameters.[14] Thus, this paper was aimed to disclose the results of the effects of this extract on serum biochemical and histological parameters in the subacute oral toxicity study.

2-MATERIAL AND METHODS
2.1-Material
2.1.1-Plant material
Fresh leaves of *Macaranga barteri* were harvested in Nangui Abrogoua University Forest (Abidjan, Côte d'Ivoire). They were identified at the National Floristic Center (CNF) of the University Felix Houphouet Boigny (Abidjan, Cote d'Ivoire) using samples kept in the national herbarium under the number 14735, April 06, 1979.

2.1.2- Animal
Experiments were conducted on male and female albino *wistar* rats (*Rattus norvegicus*). These rats were five to six weeks old and weighed between 95 and 110 g. They

were fed with IVOGRAIN® pellets and water at *libitum.* They were subjected, daily, to a temperature oscillating between 20 and 22 °C, and a photoperiod of 12h in the animal house of the Laboratory of Physiology, Pharmacology and Pharmacopoeia (LPPP) of Nangui Abrogoua University (Abidjan, Côte d'Ivoire). The different experimental protocols were followed according to the protocols of protection of experimental animals of the European Council of Legislation 87/609 / EEC.[15]

2.2- Chemicals and reagents

The chemical used were: NaCl solution (0.9%), ethanol 96 (Sigma, USA), ether (VWR International-Geldenaakfebaan464-B-3001, Leuven-Belgium); formalin (Ubipharm, France). Lab kits (Barcelona, Spain) were used for the assays of alanine aminotransferase (ALT), aspatate aminotransferase (AST), creatinine and alkaline phosphatase (ALP). Humazym test kits (Germany) and Cypress kits Diagnostics (Belgium) were used for the urea and glucose assays, respectively. Giesse Diagnostics kits (Italy) were used for the determination of calcium. Reckon Diagnostics kit (India) was used for total protein, triglyceride, sodium, chlorine, potassium, cholesterol, total and conjugated bilirubin levels determination.

2.3- METHODS

2.3.1- Preparation of the aqueous extract of the leaves of *Macaranga barteri*

Fresh leaves of *Macaranga barteri* were harvested, washed and dried in one the laboratory rooms at 25 ° C for one week. These dried leaves were powdered using a RETSCH SM 100 electric grinder (Germany). The aqueous extract was prepared according to the method described by Ehilé *et.al.*[12] Thus, one liter of distilled water was added to 100 g of *M. barteri* leaf powder and decocted for 15 min. The decoction obtained was filtered on hydrophilic cotton and on Whatman No.3 filter paper.

The solution was dried in a Friucell brand oven (Germany) at 45° C for 48 hours. The black powder obtained constituted the aqueous extract of the leaves of M. *barteri* (AEMb). The different concentrations of the extract were prepared extemporaneously by dissolving the powder in distilled water.

2.3.2- Study of the subacute toxicity of the aqueous extract of *Macaranga barteri* leaves in rats

2.3.2.1-Animals grouping

The experiments were implemented according to the OECD test guideline 407.[16] Thus, seven groups of rats were orally administered with different doses of AEMb once a day with one dose per group for 28 days. Fifty (50) rats were randomly allotted into 4 test groups of 10 rats each and a control group of 10 rats. Each group included five males and five females. The control group (group 1) received orally distilled water at 1 ml / 100 g b.w. Groups 2, 3, 4 and 5 received orally the leaves aqueous extract of *Macaranga barteri* at doses of 125, 250, 500 and 1000 mg / kg b.w. respectively. Two additional satellite groups (groups 6 and 7) of 10 rats each were added. Satelite control group (group 6) was gavaged with distilled water and satellite test group (group 7) was administrered with 1000 mg / kg b.w. of AEMb for 28 days in order to check the possible persistence or late appearance of toxic effects 14 days after the end of the treatment.

2.3.2.2- Experimental procedure

The rats in each group were gavaged using a cannula with a calculated volume of different solutions according to their body weight once a day for 28 days. After 28 days of the treatment the blood of the control and test group rats was taken from the orbital sinus of the eye and collected in dry test tubes. The animals were sacrificed by overdose of ether. The liver, heart and kidneys were removed and stored in 10% formalin for histological studies. Two weeks later, the rats in the satellite groups underwent blood samples collection and vital organs (heart, liver, and kidney) removal under the same conditions as described above.

The blood collected was centrifuged at 3000 rpm for ten min. Serum biochemical parameters were analyzed with a Prietest Touch Robonik semi automatic machine (Mahape, India). These parameters were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, conjugated bilirubin, total protein, albumin, creatinine, urea, sodium, potassium, chlorine, calcium, total cholesterol, triglycerides and glucose.

The organs kept in the 10% formalin were used for histological studies using the technique described by Hould.[17]

2 .3.3- Statistical Analysis

The statistical analysis of the data was performed using Graph Pad Prism7.00 software (San Diego, California, USA). Results were expressed as an average followed by the standard error on the mean (m±sem). One-way analysis of variance (ANOVA1) was used to verify the normality of the variables. When significant differences were revealed between the means tested, the analysis was supplemented by multiple comparisons of the mean values of the different parameters using the Turkey-Kramer test as a post test. Differences were considered statistically significat at $p < 0.05$.

3-RESULTS

3.1-Effect of aqueous leaves extract of *Macaranga barteri* on liver markers

Table 1 shows the results of the effects of AEMb administered orally to male and female rats, on liver markers. The analysis of these results revealed that AEMb induced no significant increase ($p > 0.05$) of transaminase activities (AST and ALT) in treated rats compared to control rats. Indeed, serum AST levels of male and female control rats were respectively 199 ± 7.44 and 192 ± 14.20 IU/l.

These levels ranged from 209 ± 9.90 to 214 ± 8.43 IU/l and from 207 ± 14.3 to 212 ± 10.20 IU/l, respectively, in male and female rats at AEMb doses between 125 and 1000 mg/kg b.w.

Serum ALT levels were 70.3 ± 5.77 and 67.5 ± 3.55 IU/l in male and female rats, respectively. After treatment with AEMb at studied doses, ALT levels non significantly raised between 74.5 ± 6.29 and 78.1 ± 7.34 IU/l in male rats and between 67.2 ± 3.36 and 77 ± 7.56 IU/l in female rats. As for the serum alkaline phosphatase, a non-significant increase (p> 0.05) of its activity in male and female rats treated with the studied doses of AEMb was shown, compared to control group rats. In male and female control group rats, the activity of this enzyme was respectively 413 ± 47.40 and 394 ± 34.40 IU/l while those of AEMb treated rat groups ranged from 418 ± 45.2 to 442 ± 32.2 IU/l (male rats) and from 409 ± 25.5 to 425 ± 16.2 IU/l (female rats).

With regards to the effects of AEMb doses on the total and conjugated bilirubin levels in male and female rats, the results indicated that these parameters did not undergo any significant variation (p> 0.05) when compared to the control groups. In fact, the total bilirubin values of the control groups were respectively 0.57 ± 0.13 and 0.59 ± 0.04 mg/dl.

Table 1: Effect of the Aqueous Leaves Extract of *Macaranga barteri* on Liver Markers.

Parameters	Sex	Group1 (Control distilled water)	Group 2 (AEMb 125 mg/kg b.w.)	Group 3 (AEMb 250 mg/kg b.w.)	Group 4 (AEMb 500 mg/kg b.w.)	Group 5 (AEMb 1000 mg/kg b.w.)
AST (IU/l)	M	199±7.44	209±4.29	214±8.43	210±9.00	212±10.05
	F	192±14.20	209±18.60	207±14.30	212±10.20	211±12.70
ALT (IU/l)	M	70.3±5.77	74.8±10.30	74.5± 6.29	78.1±7.34	76.6±9.46
	F	67.5±3.55	69.7±6.04	77±7.56	68.2±4.18	67.2±3.36
ALP (U/l)	M	413±47.40	418±45.20	426±55.60	433±19.10	442±32.20
	F	394±34.40	409±25.50	413±20.70	421±24.70	425±16.20
T- BILI (mg/dl)	M	0.57±0.13	0.60±0.06	0.62±0.09	0.59±0.20	0.60±0.07
	F	0.59±0.04	0.57±0.12	0.60±0.05	0.59±0.12	0.61±0.20
C-BILI (mg/dl)	M	0.18±0.02	0.17±0.05	0.15±0.03	0.17±0.09	0.2±0.08
	F	0.16±0.09	0.16±0.02	0.18±0.08	0.16±0.06	0.19±0.06
TP (mg/dl)	M	6.22±0.34	6.38±0.22	6.78±0.38	6.52±0.44	6.92±0.15
	F	5.72±0.78	6.62±0.52	6.71±0.59	7.02±0.32	6.77±0.64
Alb (g/dl)	M	2.58±0.18	2.65±0.35	2.7±0.10	2.85±0.38	2.60±0.33
	F	2.53±0.18	2.69±0.30	2.32±0.38	2.70±0.38	2.98±0.25

No significant difference (p> 0.05) was observed between the EAMb treated rats and the control groups (distilled water) for the same parameter. EAMb: Aqueous extract of the leaves of *Macaranga barteri*; number of animals per sex per group = 5, ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; T-BILI: Total bilirubin; C-BILI : Conjugated bilirubin; TP: Total proteins; Alb: Albumin; M: Male; F: Female.

Those treated with AEMb (125 -1000 mg / kg b.w.) varied between 0.59 ± 0.20 and 0.62 ± 0.09 mg/dl in male rats while in female rats those values fluctuated between 0.57 ± 0.12 and 0.61 ± 0.2 mg / dl.

Concerning conjugated bilirubin, the values of the control groups were 0.18 ± 0.02 and 0.16 ± 0.09 mg/dl, respectively in male and female rats. They reached 0.2 ± 0.08 mg / dl in male rats and 0.19 ± 0.06 mg /dl in female group when treated with AEMb at 1000 mg/kg b.w.

The results also revealed that the levels of total proteins and albumin in the rats treated with the studied doses of AEMb were not significantly changed compared to the values of the control groups. Indeed, the total protein control values which were 6.22 ± 0.34 and 5.72 ± 0.78 mg /dl varied to attain 6.92 ± 0.15 and 7.02 ± 0.32 mg/dl respectively in male and female rats. Serum albumin values of the male and female control rats were 2.58 ± 0.18 and 2.53 ± 0.18 g/dl respectively. These levels reached 2.85 ± 0.38 and 2.98 ± 0.25 g / dl respectively in male and female rats after treatment with AEMb.

3.2-Effect of the aqueous leaves extract of *Macaranga barteri* on the electrolytes and kidney markers in rats

The effects of the AEMb on electrolytes and renal markers are presented in **table 2**. AEMb increased non significantly (p> 0.05) the serum sodium levels from 120 ± 2.81 (control) to 135 ± 3.9 mmol /l in male rats and from 124 ± 1.48 (control) to 137 ± 6.70 mmol/ l in female rats.

In contrast, the serum potassium levels, which were 7.06 ± 0.21 and 7.35 ± 0.33 mmol/ l, respectively in male and female rats diminished non significantly (p> 0.05) in male and female rats treated with AEMb. In fact, potassium levels reached 6.35 ± 0.46 (250 mg/kg b.w.) and 6.49 ± 0.27 mmol/ l (125 mg/kg b.w) respectively in male and female rats.

As for the serum calcium levels, the baseline was 12.00 ± 1.29 and 12.15 ± 0.89 mmol/l respectively in male and female rats. No significant variation (p> 0.05) was observed after AEMb treatment compared to rats in the control group.

The results of the effect of the AEMb on the rats serum chlorine levels indicated a non-significant increase (p> 0.05) at extract doses between 125 and 500 mg / kg b.w. Control values that were 115 ± 5.44 and 113 ± 3.76 mg/dl non significantly augmented to 136 ± 2.70 and 141 ± 5.17 mg / dl respectively in male and female rats (500 mg / kg b.w.). However, the dose of 1000 mg / kg b.w. of the extract induced a significant increase (p <0.05) of this marker which reached 154 ± 6.12 and 161 ± 2.26 mg / dl respectively in male and female rats.

Table 2: Effects of the aqueous leaf extract of *Macaranga barteri* on electrolytes and renal serum markers

Parameters	Sex	Group1 (Control distilled water)	Group 2 (AEMb 125 mg/kg b.w.)	Group 3 (AEMb 250 mg/kg b.w.)	Group 4 (AEMb 500 mg/kg b.w.)	Group 5 (AEMb 1000 mg/kg b.w.)
Sodium (mmol/l)	M	120±2.81	128±3.17	133±4.16	134±3.26	135±3.9
	F	124±1.48	130±4.62	131±4.58	133±3.40	137±6.70
Potassium (mmol/l)	M	7.06±0.21	6.75±0.24	6.35±0.46	6.41±0.27	6.38±0.34
	F	7.35±0.33	6.49±0.27	6.78±0.35	7.11±0.21	6.96±0.37
Calcium (mmol/l)	M	12.00±1.29	12.10±1.17	12.32±2.19	12.50±1.99	12.00±1.29
	F	12.15±0.89	12.20±1.56	12.32±2.12	12.00±1.36	12.65±0.78
Chlorine (mg/dl)	M	115±5.44	122±3.28	133±2.53	136±2.70	154±6.12*
	F	113±3.76	125±4.46	129±3.28	141±5.17	161±2.26*
Urea (mg/dl)	M	60.6±3.01	61.7±6.24	63.3±3.67	66.2±3.79	79.1±5.79*
	F	61.4±3.66	62±5.41	64.3±3.37	67.4±3.78	80.8±3.82*
Creatinine (mg/dl)	M	0.62±0.21	0.63±0.09	0.67±0.07	0.65±0.15	0.68±0.05
	F	0.61±0.13	0.65±0.05	0.69±0.10	0.68±0.07	0.69±0.06

* p <0.05: Significant increase compared to control group.

AEMb: Aqueous extract of the leaves of *Macaranga barteri*; M: Male; F: Female; number of animals per sex per group= 5.

With respect to the effect of AEMb on the serum urea levels, the results disclosed a non significant (p> 0.05) and dose-dependent increase in serum urea at 125, 250, 500 mg / kg b.w. of AEMb compared to the control rats. In male rats, the control value of urea, which was 60.60 ± 3.01 mg / dl attained 66.20 ± 3.79 mg/dl at AEMb dose of 500 mg / kg b.w. That in female rats increased from 61.40 ± 3.66 mg / dl (control group) to 67.40 ± 3.78 mg / dl (500 mg/kg b.w).

Yet the dose of 1000 mg / kg b.w. of AEMb triggered a significant (p <0.05) augmentation in the urea level of the male and female treated rats compared to the control rats by increasing these values to 79.10 ± 5.79 and 80.8 ± 3.82 mg / dl respectively.

Serum creatinine levels were non-significantly increased (p >0.05) following repeated administration of AEMb at doses ranging from 125 to 1000 mg / kg b.w. compared to rats in the control groups. In fact, the creatinine level values of the male group ranged from 0.62 ± 0.21 (control) to 0.68 ± 0.05 mg / dl (AEMb 1000 mg / kg b.w.), and those of the female rats varied from 0.61 ± 0.13 (control) to 0.69 ± 0.06 mg / dl (AEMb 1000 mg / kg b.w.).

3.3-Effect of the aqueous leaves extract of *Macaranga barteri* on some lipid profile and glycemia in rats

The results of the effects of studied doses of AEMb on rats' serum triglycerides, total cholesterol and glycemia are shown in **Table 3.**

The different doses of AEMb had no significant effect on serum levels of the parameters mentioned above. Indeed, triglyceride rates did not vary significantly (p> 0.05) in both sexes as compared to control groups (0.88 ± 0.26 g/l male and 0.96 ± 0.12 g/l in female rats).

Total cholesterol rates non significantly diminished from 1.98 ± 0.32 and 2.06 ± 0.12 g/l (respectively male and female control rats) to 1.65 ± 0.11g/l (male rats) and 1.74 ± 0.16 g/l (female rats) at AEMb 1000 mg/kg b.w.

As regards blood glucose rates, non significant reductions were recorded from control groups to AEMb 1000 mg/kg b.w. groups in male (0.86 ± 0.15 to 0.6 8 ± 0.16 g/l) and female (0.89 ± 0.16 to 0.69 ± 0.12 g/l) rats.

3.4- Effect of the aqueous leaves extract of *Macaranga barteri* on the histology of certain vital organs in rats

Sections of organs (liver, kidneys and heart) of the control rats and those gavaged with the different doses of *M. barteri* of the male and female rats are dipicted by the photomicrographs in **Fig 1 and Fig 2.** The results indicated that AEMb did not cause any damage to these organs.

Table 3: Effect of the aqueous leaves extract of *Macaranga barteri* on the lipid profile and blood glucose levels in rats.

Parameters	Sex	Group1 (Control distilled water)	Group 2 (AEMb 125 mg/kg b.w.)	Group 3 (AEMb 250 mg/kg b.w.)	Group 4 (AEMb 500 mg/kg b.w.)	Group 5 (AEMb 1000 mg/kg b.w)
Trigly (g/l)	M	0.88±0.26	0.82±0.17	0.89±0.19	0.92±0.11	0.84±0.14
	F	0.96±0.12	1.02±0.22	0.89±0.26	0.86±0.25	0.89±0.25
T-Chol (g/l)	M	1.98±0.32	1.75±0.09	1.70±0.11	1.68±0.05	1.65±0.11
	F	2.06±0.12	2.00±0.28	1.96±0.86	1.82±0.1	1.74±0.16
Gly (g/l)	M	0.86±0.15	0.75±0.04	0.74±0.07	0.70±0.14	0.68±0.16
	F	0.89±0.16	0.77±0.05	0.75±0.06	0.76±0.25	0.69±0.12

T-Chol: Total cholesterol; Trigly: Triglycerides; Gly: Glycemia; M: Male; F: Female; AEMb: Aqueous extract of the leaves of *Macaranga barteri*; number of animals per sex per group= 5

No statistically significant difference (P> 0.05) was observed between groups of rats treated with AEMb and those of control groups (distilled water).

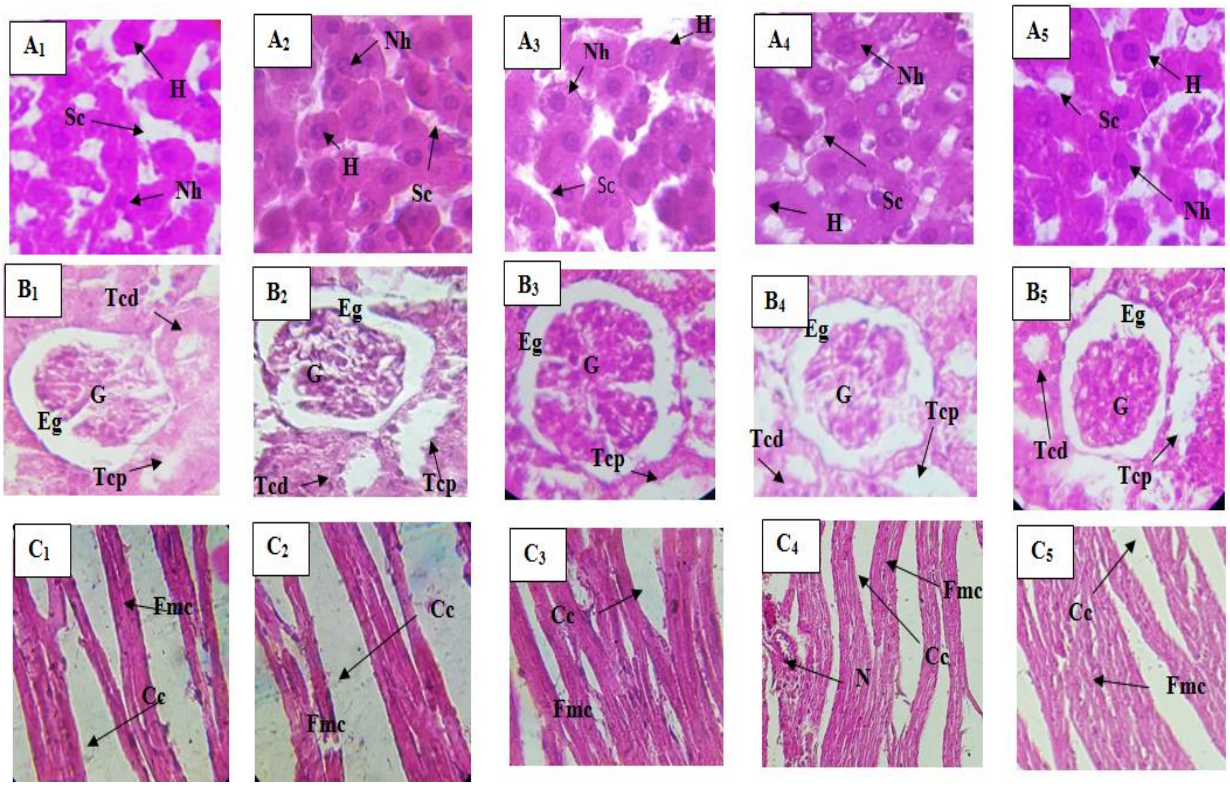

Fig 1: Photomicrographs of histological sections of the liver, kidneys and heart of experimental male rats.

A_1 to A_5 : hepatic tissues: control rats (**A_1**), rats treated with AEMb 125 mg / kg b.w. (**A_2**), rats treated with AEMb 250 mg / kg b.w. (**A_3**), rats treated with AEMb 500 mg / kg b.w. (**A_4**): rat treated with AEMb 1000 mg / kg b.w. (**A_5**); **B_1 to B_5:** kidney tissues: control rats (**B_1**), rats treated with EAMb 125 mg/kg b.w. (**B_2**), rats treated with AEMb 250 mg / kg b.w. (**B_3**), rats treated with AEMAb 500 mg / kg b.w. (**B_4**), rats treated with EAMb 1000 mg / kg b.w. (**B_5**); **C_1 to C_5**: heart tissues:control rats (**C_1**), rats treated with AEMb 125 mg / kg b.w. (**C_2**), rats treated with AEMb 250 mg / kg b.w. (**C_3**), rats treated with AEMb 500 mg / kg b.w. (**C_4**), rats treated with AEMb 1000 mg / kg b.w. (**C_5**), Hematoxylin-Eosin staining; G X 1000**;** **H**: hepatocytes; **Nh**: hepatocyte nucleus; **Sc**: capillary sinusoid **G**: glomerulus; **Eg**: glomerular space; **Tcp**: proximal convoluted tubule; **Tcd**: distal convoluted tubule; **Fmc**: cardiac muscle fibers; **Cc**: cardiac cavity; **N**: heart cell nucleus

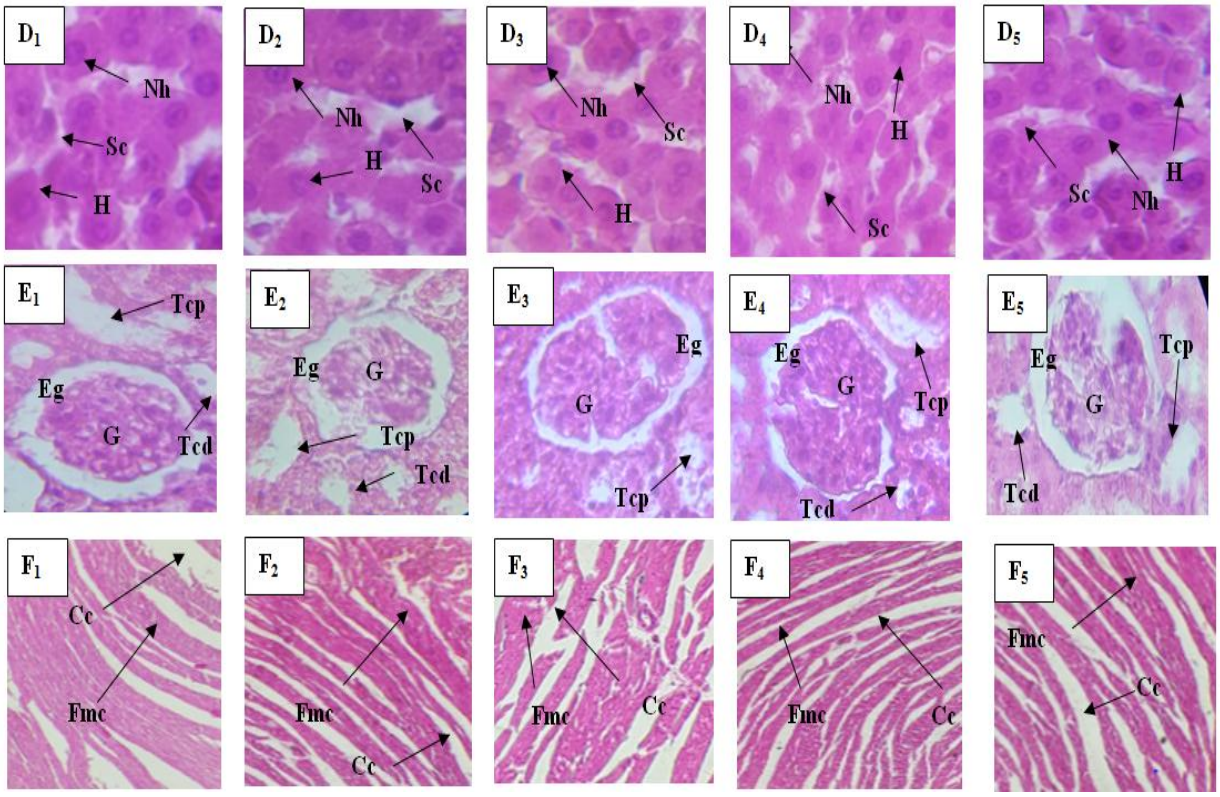

Fig 2: Photomicrographs of histological sections of the liver, kidneys and heart of experimental female rats.

D_1 to D_5 : hepatic tissues: control rats (**D_1**), rats treated with AEMb 125 mg / kg b.w. (**D_2**), rats treated with AEMb 250 mg / kg b.w. (**D_3**), rats treated with AEMb 500 mg / kg b.w. (**D_4**): rats treated with AEMb 1000 mg / kg b.w. (**D_5**); **E_1 to E_5:** kidney tissues: control rats (**E_1**), rats treated with EAMb 125 mg/kg b.w. (**E_2**), rats treated with AEMb 250 mg / kg b.w. (**E_3**), rats treated with AEMAb 500 mg / kg b.w. (**E_4**), rats treated with EAMb 1000 mg / kg b.w. (**E_5**); **F_1 to F_5**: heart tissues:control rats (**F_1**), rats treated with AEMb 125 mg / kg b.w. (**F_2**), Rats treated with AEMb 250 mg / kg b.w. (**F_3**), rats treated with AEMb 500 mg / kg b.w. (**F_4**), rats treated with AEMb 1000 mg / kg b.w. (**F_5**). Hematoxylin-Eosin staining; G X 1000**;** **H**: hepatocytes; **Nh**: hepatocyte nucleus; **Sc**: capillary sinusoid **G**: glomerulus; **Eg**: glomerular space; **Tcp**: proximal convoluted tubule; **Tcd**: distal convoluted tubule; **Fmc**: cardiac muscle fibers; **Cc**: cardiac cavity.

3.4-Reversible or delayed side effects of the aqueous leaves extract of *Macaranga barteri* on biochemical markers

The results of reversible or delayed effects of AEMb on biochemical markers are summarized in **Table 4, 5 and 6.** Two weeks after the end of the different treatments, no significant variation ($p>0.05$) in serum levels of the different biochemical parameters of male and female rats treated with 1000 mg/kg b.w. of AEMb was recorded. Thus, no delayed effect was found out. In addition, the disturbances caused by the administration of 1000 mg/kg b.w. of the extract on serum chlorine and urea levels disappeared two weeks after the end of treatment. Therefore the toxic effect of AEMb on these renal markers were transient.

3.5 -Reversible or delayed effects of the aqueous leaves extract of *Macaranga barteri* on the histology of certain vital organs in rats

Histological sections of male and female rat liver, kidney and heart two weeks after cessation of administration of AEMb (satellite groups) are illustrated in Fig 3. These photographs showed no abnormality of architecture sections of organs of the satellite rats groups compared to those of control rats.

Table 4: Effects of the aqueous extract of the leaves of *Macaranga barteri* on liver markers two weeks after the end of treatment.

Parameters	Sex	Two weeks after the end of the treatment	
		Group 6 (Control Distilled water)	Group 7 (AEMb 1000 mg/kg b.w.)
AST (IU/l)	M	198±11.90	201±14.1
	F	203±13.20	206±10.6
ALT (IU/l)	M	68.6±10.20	72.8±9.19
	F	64.3±6.76	68.00±5.18
ALP (IU/l)	M	370±54.9	353±16.50
	F	392±12.5	378±40.2
T-BILI (mg/dl)	M	0.65±0.05	0.60±0.07
	F	0.52±0.06	0.53±0.09
C-BILI (mg/dl)	M	0.187±0.02	0.170±0.01
	F	0.180±0.03	0.189±0.02
TP (mg/dl)	M	5.98±0.44	5.90±0.16
	F	6.35±0.11	6.13±0.2
ALB (g/dl)	M	2.71±0.17	2.67±0.21
	F	2.52±0.2	2.62±0.14

ALT: Alanine transaminase; **AST**: Aspartate transaminase; **ALP**: Alkaline phosphatase; **T-BILI**: Total bilirubin; **C-BILI :** Conjugated bilirubin; **TP**: Total proteins; **Alb:** Albumin; **M**: Male; **F:** Female.

No significant difference (p> 0.05) was observed between the AEMb treated rats and the control groups (distilled water) for the same parameter. **AEMb**: Aqueous extract of the leaves of *Macaranga barteri*; number of animals per sex per group = 5.

Table 5: Effects of the aqueous extract of the leaves of *Macaranga barteri* on electrolytes and renal serum markers two weeks after the end of treatment.

Parameters	Sex	Two weeks after the end of the treatment	
		Group 6 (Control Distilled water)	Group 7 (AEMb 1000 mg/kg b.w.)
	F	2.52±0.2	2.62±0.14
Sodium (mmol/l)	M	128±3.6	133±2.06
	F	126±7.28	138±5.40
Potassium (mmol/l)	M	6.35±1.31	6.88±2.65
	F	6.67±0.53	7.07±0.29
Calcium (mmol/l)	M	11.80±2.62	12.12±3.12
	F	11.30±1.02	12.50±2.15
Chlorine (mg/dl)	M	113±3.35	117±3.20
	F	112±2.18	114±4.48
Urea (mg/dl)	M	60.42±5.33	61.20±4.90
	F	60.8±2.14	62.7±4.39
Creatinine (mg/dl)	M	0.61±0.09	0.63±0.15
	F	0.62±0.12	0.64±0.017

No significant difference (p> 0.05) was observed between the AEMb treated rats and the control groups (distilled water) for the same parameter. **AEMb**: Aqueous extract of the leaves of *Macaranga barteri*; number of animals per sex per group = 5; **M:** Male; **F:** Female.

Table 6: Effects of the aqueous extract of the leaves of *Macaranga barteri* on the lipid profile and blood glucose levels two weeks after the end of treatment.

Parameters	Sex	Two weeks after the end of the treatment	
		Group 6 (Control Distilled water)	Group 7 (AEMb 1000 mg/kg b.w.)
Trigly (g/l)	M	0.85±0.13	0.83±0.20
	F	0.82±0.10	0.80±0.15
T-Chol (g/l)	M	1.92±0.22	1.79±0.62
	F	1.80±0.10	1.74±0.14
Gly (g/l)	M	0.76±0.21	0.74±0.26
	F	0.77±0.31	0.75±0.19

No significant difference (P> 0.05) was observed between the AEMb treated rats and the control groups (distilled water) for the same parameter. **AEMb**: Aqueous extract of the leaves of *Macaranga barteri*; number of animals per sex per group = 5; **T-Chol :** Total cholesterol; **Trigly:** Triglycerides; **Gly:** Glycemia; **M:** Male; **F:** Female

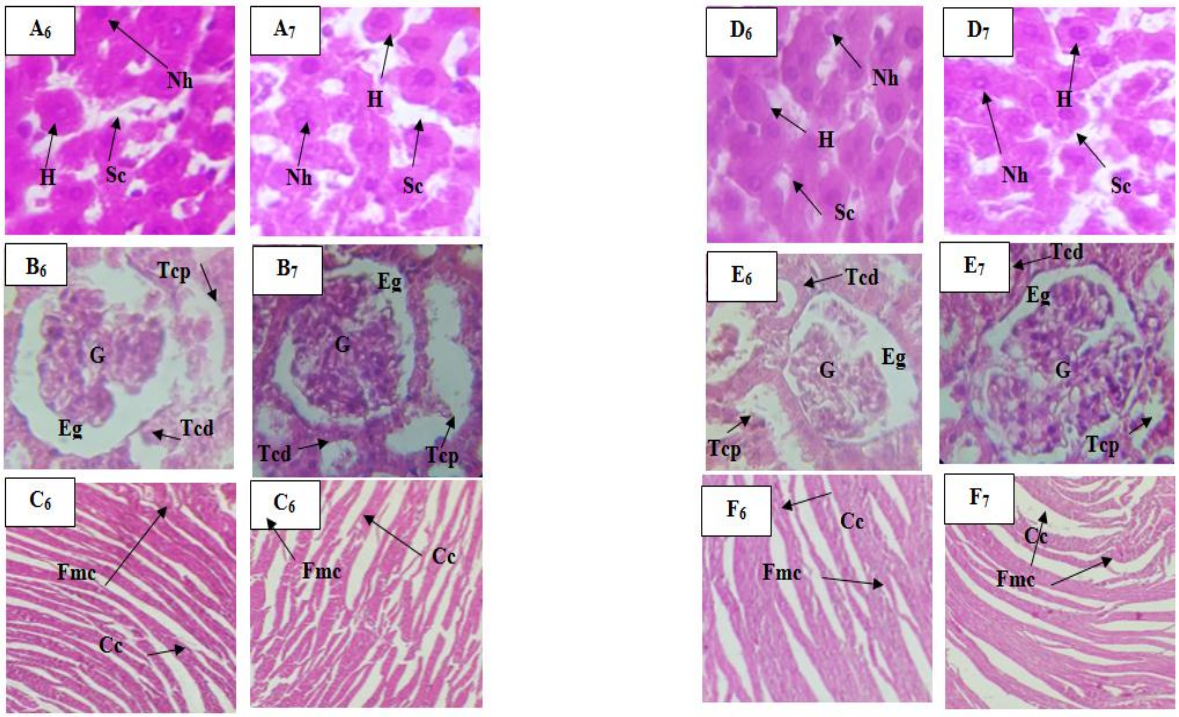

Fig 3: Microphotographs of histological sections of male and female rat liver, kidneys and heart two weeks after the end of treatment.

A_6 : Male rat liver section of the satellite control, **A_7 :** Male rat liver section of the satellite test group, **B_6 :** Male rat kidney section of the satellite control, **B_7 :** Male rat kidney section of the satellite test group, **C_6 :** Male rat heart section of the satellite control, **C_7 :** Male rat heart section of the satellite test group; **D_6 :** Female rat liver section of the satellite control, **D_7 :** Female rat liver section of the satellite test group, **E_6 :** Female rat kidney section of the satellite control, **E_7 :** Female rat kidney section of the satellite test group, **F_6 :** Female rat heart section of the satellite control, **F_7 :** Female rat heart section of the satellite test group. Hematoxylin-Eosin staining; G X 1000; **H:** hepatocytes; **Nh:** hepatocyte nucleus; **Sc**: capillary sinusoid **G:** glomerulus; **Eg:** glomerular space; **Tcp:** proximal convoluted tubule; **Tcd:** distal convoluted tubule, **Fmc:** cardiac muscle fibers; **Cc**: heart chamber.

DISCUSSION

The results obtained during the subacute toxicity study of the aqueous leaves extract of *Macaranga barteri* (AEMb) did not reveal any significant changes in serum hepatic, renal, electrolytes, lipids and glucose of rats treated with AEMb. However, significant increases in serum chlorine and urea levels were recorded in both male and female rats treated with 1000 mg/ kg b.w. of AEMb.

In fact, the study of serum parameters of liver function showed no significant difference in AST, ALT and ALP compared to control rats. Under normal conditions, these

enzymes are found in hepatic, cardiac and renal cells. But when these organs are damaged, the enzymes are found in the bloodstream.[18] The increase of transaminases in the serum testifies either the destruction of the tissues or the modification of membrane permeability.[19] Hepatic function is also appreciated by the determination of total and conjugated bilirubin, total protein and albumin. Serum levels of these parameters remained normal in the AEMb treated rats groups. Normal levels of albumin and total protein in the serum may imply that the secretory functions of the liver have not been affected.[20] AEMb has no adverse effects on liver parameters. These results are similar to those obtained by Adewale et al.[21] and Da Silva et al.[22] with the aqueous extracts of the leaves of *Crassocephalum rubens and Serjania marginata*. Indeed, these authors demonstrated that the administration for twenty-eight days of different extracts at doses between 250 and 1000 mg / kg b.w. did not affect all the liver parameters in rats.

With regards to renal parameters, creatinine and urea are considered as the main ones. Their increase or decrease reflects a dysfunction of renal function.[23] The results of this study exhibited a non-significant increase in serum creatinine and urea concentrations after treatment with 125 to 500 mg/kg b.w. of AEMb. However, hyperuremia was observed after 28 days of treatment with AEMb at 1000 mg/kg b.w. compared to control groups in both male and female rats. This increase in serum urea level could be explained by an increase of the catabolism of protein compound and impaired renal filtration mechanism function.[24] Repeated administration of AEMb at 1000 mg / kg b.w. could influence the ability of the kidneys to excrete this metabolite.The effects of EAMb are similar to those of methanol extract of *Lycopersicon esculentum* leaves. According to a study conducted by Nguenang et al.[25] methanol extract of *L. esculentum* leaves at doses between 250 and 1000 mg / kg b.w. had no significant effect on rat creatinine level whereas a significant increase in urea levels in both male and female rats was noticed.

Serum electrolytes intervene in the coagulation process and the maintenance of the hydration of the organism. Their excretion is ensured by the kidney. An abnormal concentration of electrolytes is a sign of renal impairment.[26] In this study, there was no significant changes of serum calcium, sodium and potassium levels except chlorine which significantly increased in rat treated with AEMb at 1000 mg/kg b.w. The significant increase in chlorine suggests that AEMb could interfere with the hydration system of the rats. Indeed, Akers and Denbow[27] reported that chlorine plays a key role in osmotic pressure between extracellular and intracellular compartments. An increase in chlorine level may involve renal tubule functioning.[28] These results are similar to those of Sharaibi et al[29] who showed that the aqueous extract of the leaves of *Nymphaea lotus* did not cause any change in the serum calcium, sodium and potassium concentrations. However, an increase in chlorine level was observed at the dose of 400 mg/ kg b.w. after 28 days of treatment in rats.

Regarding the lipid profile, AEMb resulted in a non-significant decrease in cholesterol levels in male and female rats and it did not influence the serum triglyceride levels. The decrease in serum total cholesterol levels, even if not significant, may be beneficial. It may be due to the presence of secondary metabolites including polyphenols and flavonoids[12] which are endowed with anti-hyperlipidemic properties.[30] According to Cohen,[31] the decrease in plasma cholesterol is due to a problem of micelle and bile acid formation in the digestive tract interfering with the absorption of total cholesterol and its excretion. This result is similar to those of. Donkor et al.[32] who observed no change in serum lipid parameters (cholesterol and triglycerides) in rats gavaged with 500 mg/kg b.w. of hydroethanolic leaves extract of *Duranta erecta* for 28 days. As for blood glucose level, the repeated administration for 28 days of AEMb did not elicit any significant variation. This result is similar to those obtained by Ribeiro et al.[33] Indeed, these authors revealed that the extract of *Chrysobalanus icaco*, at doses between 100 and 400 mg / kg b.w. did not induce any change in blood glucose levels in rats.

The administration of AEMb showed no abnormalities in hepatic, cardiac and renal tissues. Thereby it can be deduced that this extract is not dangerous for the structural organization of these organs.

These results are similar to those of Balaji and Ganesan[34] who indicated that repeated administration of the hydroalcoholic extract of *Caryota urens* leaves in rats, did not provoke any destruction of the liver, heart and kidneys architecture of treated rats compared to the control. Two weeks after the end of treatment with AEMb at 1000 mg/kg b.w. the disturbances observed during the treatment disappeared and no delayed effect was noticed. This suggests that the potential toxic effects of AEMb at 1000 mg/kg b.w. on renal function are reversible. These results corroborate those of Tchokomeni et al.[35] with the aqueous extract of the leaves of *Eremomastax speciosa*.

CONCLUSION

This study showed that the aqueous extract of *M. barteri* did not cause any disturbance of the serum hepatic biochemical parameters (alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total proteins, albumin, total and conjugated bilirubins) some of the renal serum biochemical parameters (creatinine, sodium, potassium, and calcium), some lipid parameters (total cholesterol and triglycerides) and glucose in rats. However, a significant increase was observed in the levels of chlorine and urea in male and female rats treated with 1000 mg/kg b.w of EAMb. Therefore, this extract could affect kidney functioning at high dose of 1000 mg/kg b.w.

Nevertheless, these effects on renal impairment were transient because they vanished two weeks after the end of treatment with AEMb. The histological study revealed no abnormalities of the rats' liver, kidney, and heart when treated with any studied doses of AEMb.

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Conflict of Interest
The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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