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Original Article

Effect of aqueous extract of *Terminalia superba* Engl. et Diels (Combretaceae) stem bark on the biochemical parameters of *Ratus norvegicus.*

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ABSTRACT

Terminalia superba Engl. and Diels (Combretaceae) is a medicinal plant used for the treatment of gastric ulcer in Côte d'Ivoire. In order to verify the safety of this plant, each day, during 28 days, aqueous extract of the stem bark is administered orally to three groups of rats at doses 125, 250 and 500 mg / kg body weight. The control group received distilled water. Every week, rats blood samples collected into plain tubes were used to determine the biochemical parameters such as transaminases AST and ALT, urea, creatinine, triglycerides, glycaemia and total cholesterol by enzymatic and kinetic methods. Results showed that at the fourth week, the doses 250 and 500 mg / kg body weight induced a significant decrease in urea and triglycerides and a significant increase in total cholesterol. Concerning ALT, AST, creatinine and glycaemia, no significant variation was observed. The use of aqueous extract of *Terminalia superba* stem bark would not be toxic to the liver and kidneys at the doses 125 to 500 mg / kg body weight.

Keywords: *Terminalia superba*, safety, biochemical parameters, *Ratus norvegicus*.

INTRODUCTION

The use of traditional medicine plants for the treatment of various diseases is experiencing a rapid and global growth. This interest for the medicinal plants in primary health care is attributed to the easy access, efficiency and low cost of the herbal remedies [**8**]. *Terminalia Superba* is a tree belonging to the family of Combretaceae and measuring up to 40 m high [**6**]. The decoction of the stem bark of this plant is used for treating women infertility [**2**].. The infused of *Terminalia superba* stem bark is used against gastric ulcers, malaria and measles [**4,15**]. Studies have revealed that the aqueous extract of *Terminalia superba* stem bark has anti gastric ulcer properties. These same studies have shown that this extract has no acute toxicity at the dose 6000 mg / kg body weight [**17**].

Despite the widespread use of this plant in traditional medicine, few studies refer to the risks associated with the repeated administration prolonged use.

This study aims at evaluating the oral subacute toxicity of the aqueous extract of *Terminalia superba* stem bark for 28 days on the blood biochemical parameters of rats.

MATERIALS AND METHODS

Plant material: The stem bark of *Terminalia superba* were collected in December 2013 in Ebilassokro a village of Abengourou located about 210 km from Abidjan (Côte d'Ivoire). They were identified through samples stored under the respective 2456 numbers 4 June 1954, 26 March 4207 1957 10477 of 26 February 1969 and 416 of 3 April 1974 at the National Herbarium of Côte d'Ivoire. The barks were authenticated elsewhere by late Professor Ake Assi National Centre of Floristic (CNF) of the Félix Houphouët Boigny University of Cocody, Abidjan (Côte d'Ivoire).

Animal material: The research was conducted on albino rats of the species *Ratus norvegicus* belonging to wistar strain with ages between 3 and 8 weeks, and having a body mass between 100 and

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136 g. These animals were fed with pellets from FACI® and willingness to tap water. They were subjected daily to the ambient temperature of room, 12 hours of light and 12 hours of darkness.

Preparation of the aqueous extract of *Terminalia superba* stem bark: The extraction method is based on the traditional method of preparation of *Terminalia superba.* The bark is washed with distilled water, cut into small pieces and dried in the laboratory room at 27°C for two weeks and then pulverized using a mortar.

Fifty (50) grams of this powder are infused for 15 minutes in 1 liter of boiled distilled water. The aqueous solution obtained is filtered through cotton wool and then Whatman filter paper N° 1. Half of a liter of boiled distilled water is added to the marc which undergoes a second infusion for 15 minutes. This is also infused and filtered. The filtrates were evaporated and dried in an oven at 45 ° C for 48 hours. A powder weighing 22.56 g of brown black color that will help prepare the aqueous extract of *Terminalia superba* stem bark (AETS).

Subacute toxicity studies: This subacute toxicity study was done in compliance with the OECD guideline N° 407 [**20**]. It consisted of daily oral administrations of doses of the extract to three groups of rats for 28 days. Forty (40) rats were divided into four groups of 10 animals including three (3) test groups and a control group. Each group consists of five (5) males and five (5) females. Three doses were prepared according to previous works [17]. The groups B, C and D received respectively the doses 125, 250 and 500 mg / kg body weight of the extract. The group A received distilled water. The volume administered was done by force-feeding at 2 ml / 100g body weight. Before administering different doses of the extract, the animals in each group were individually marked and weighed.

Blood biochemical examination: At the end of each week, the animals fasted the night before, were anesthetized with ether and blood is collected early in the morning thinks to the amputation technique of the tip of the tail. It is to cut 5 mm from the tip of the tail with scissors, after the tail is disinfected with alcohol at 96° [1**8**]. Glycaemia is determined directly from whole blood using a glucometer Accu-Chek® (Roche Diagnostics) according to glucose oxidase method [**23**]. 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 some other biochemical parameters. Alanine transferase (ALT) and aspartate transferase (AST) have been determined by the kinetic method [**12**]. The determination of serum total cholesterol and urea was perform by the enzymatic method [**5,14,22**]. Creatinine is determinated using the colorimetric method [**9**]. Triglycerides was also assayed by the colorimetric method [10].

Statistical analyses: The values are presented as the mean followed by the standard error on the mean (M ± SEM). The comparisons of averages are performed relative to the control, with the repeated measures ANOVA. When significant differences were found between the control and the tests groups, ANOVA is complemented by the multiple comparisons test of means values using the test of Turkey-Kramer. The differences are significant for p value 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.

RESULTS

On Renal serum markers, significant changes were observed (Table I). Thus total aqueous extracts of *Terminalia superba* administered to rats indicated that the urea of groups B, C and D is less than the rate of control group during the 4 weeks of the study. This reduction is significant ($p < 0.05$) during the four weeks at the doses 250 and 500 mg / kg body weight. The variations of the creatinine levels are not significant ($p > 0.05$) throughout the experiment (table 1).

Regarding the hepatic serum markers, the aqueous extract of *Terminalia superba* was no change (table2). Indeed, the values of transaminases ALT of the test groups have not been disrupted significantly ($p > 0.05$) compared to the control group. In terms of transaminases AST, a non-significant ($p > 0.05$) average values of the test groups compared to the control group was observed. The glycaemia levels of the rats of the test groups was not disrupted ($p > 0.05$) compared to the control group (table 3). But the level of serum triglycerides was lower than the control group during the 4 weeks of treatment. This reduction is significant ($p < 0.05$) in the fourth week of treatment at the doses 250 and 500 mg / kg body weight. As for total cholesterol, the rate of the test groups is above that of the control over the four weeks. At the fourth week, this increase is significant ($p < 0.05$) at the doses 250 and 500 mg / kg body weight.

DISCUSSION

Urea and creatinine are biochemical markers of renal function and reflects a significant increase renal dysfunction [**13**]. Our results showed that

serum urea of the rats treated with the extract were low for the doses of 250 and 500 mg / kg body weight in the fourth week. Suggesting that our extract is not nephrotoxic. These results are contrary to those obtained by others authors who observed a significant increase in serum concentration of urea in the fourth week with the aqueous extract of stem bark of *Spondias mombin* (Anacardiaceae) at doses of 500 and 1000 mg / kg body weight [**11**]. According to others studies**,** creatinine exclusively related to glomerular filtration, is a evidence to the evaluation of renal function more than serum urea [**7**]. The results of our work revealed no significant increase in serum creatinine concentration throughout the four weeks of study. Thus the aqueous extract of *Terminalia superba* does not cause nephrotoxicity at doses used for 28 days. Our extract will not have any effect on the structure and functioning of the kidneys. These results are contrary to those obtained by the authors who report that the ethanol extract of the leaves of *Chrysophyllum Albidum* (Sapotaceae) significantly reduced serum creatinine in rats at doses of 1000 and 1500 mg / kg body weight [**1**]. Serum transaminases are most often used to assess liver function. ALT and AST are the main enzymes used to assess liver function[**24**]. The increase of these enzymes in the blood usually indicate liver damage that can lead to obstruction of the intrahepatic bile ducts, primary biliary cirrhosis or disruption of hepatic architecture [**3**]. Our study has shown no significant increase of these enzymes in the experiments. Our results are similar to those obtained in the study which showed that the aqueous extract of *Mormodica charantia* (Cucurbitaceae) did not disrupt the enzymatic activity of transaminases ALT and AST [**21**]. The administration of the total aqueous extract of *Terminalia superba* stem bark would not affect liver function in rats at doses used. The aqueous extract of *Terminalia superba* stem bark does not affect glycaemia levels. These results are similar to those observed during the administration of the aqueous extract of the leaves of *Parkia biglobosa* (Mimosaceae) in rabbits. This study related that the aqueous extract of the leaves of *Parkia biglobosa* stabilizes glycaemia [**25**]. An increase in total cholesterol and triglyceride levels is a risk factor for cardiovascular disease [**19**]. Regarding the lipid profile, our extract lowers triglyceride levels and increases the total cholesterol in the lots treated with doses of 250 and 500 mg / kg body weight compared to that of the control in the fourth week. Our results are contrary to those obtained with the aqueous extract of *Sacoglottis gabonensis* stem bark. This study indicate that no significant changes in serum triglycerides and total cholesterol after the oral administration of this extrat to rats for 28 days at the doses 3.5 ; 35 and 350 mg / kg body weight[**16**].

CONCLUSION

These studies have shown that the aqueous extract of *Terminalia superba* stem bark has no toxic effect on renal and hepatic function. However, its long-term use may disrupt the lipid metabolism.

Table 1: Effect of aqueous extract of *Terminalia superba* stem bark on the kidney serum markers

Parameters	Doses (mg/kg bw.)	Week 1	Week 2	Week 3	Week 4
Urea (g/l)	0	0.84±0.035	1.08±0.044	0.57±0.039	0.86±0.076
	125	0.78±0.044	0.95±0.043	0.51±0.048	0.78±0.035
	250	0.72±0.036	0.93±0.022	0.49±0.580	**0.67±0.020[a]**
	500	0.77±0.051	0.92±0.048	0.55±0.036	**0.67±0.086[a]**
Creatinine (mg/dl)	0	8.17±0.249	8.34±0.953	8.93±0.902	11.40±0.372
	125	8.48±0.758	8.66±0.845	8.56±0.524	11.80±0.328
	250	8.57±0.448	8.93±0.881	9.28±0.554	11.60±0.658
	500	8.14±0.770	8.91±0.872	9.07±0.789	11.20±0.611

n=10/ group.

Table 2: Effect of aqueous extract of *Terminalia superba* stem bark on the liver serum markers

Parameters	Doses (mg/kg body weight)	Week 1	Week 2	Week 3	Week 4
ALT (Ul/L)	0	10.30±0.715	10.10±0.738	10.20±0.792	10.40±0.649
	125	10.40±0.653	10.50±0.824	10.40±0.596	10.60±0.603
	250	10.50±0.764	10.60±0.596	10.60±0.649	10.70±0.782
	500	10.70±0.606	10.80±0.703	10.70±0.667	10.80±0.727
AST (Ul/L)	0	38.70±0.882	37.90±1.220	37.30±0.421	37.30±1.440
	125	36.30±1.380	36.80±0.701	34.90±0.583	36.70±0.494
	250	37.10±1.110	37.40±0.997	36.30±1.210	37.60±0.649
	500	35.30±1.280	35.70±0.969	35.70±0.494	35.30±0.882

n=10/ group.

Table 3: Effect of aqueous extract of *Terminalia superba* stem bark on the others serum markers

Parameters	Doses (mg/kg body weight)		Week 1	Week 2	Week 3	Week 4
Glycaemia (g/l)	0		1.11±0.052	1.08±0.056	0.66±0.038	1.01±0.044
	125		1.11±0.040	1.20±0.071	0.76±0.036	1.08±0.101
	250		1.15±0.061	1.15±0.068	0.77±0.026	1.15±0.068
	500		1.05±0.043	1.01±0.019	0.72±0.029	1.14±0.034
Triglycerides (g/l)	0		2.08±0.234	2.54±0.253	1.84±0.274	3.20±0.215
	125		1.85±0.273	1.98±0.238	1.55±0.222	2.89±0.206
	250		1.76±0.253	1.98±0.287	1.47±0.237	**2.27±0.249[a]**
	500		1.53±0.227	1.75±0.257	1.51±0.219	**2.17±0.254[a]**
Total Cholesterol (g/l)	0	A	1.80±0.303	2.36±0.282	2.37±0.44	2.29±0.486
	125	B	2.09±0.442	2.69±0.409	3.16±0.342	3.10±0.300
	250	C	1.98±0.249	2.52±0.395	3.53±0.157	**3.60±0.222[b]**
	500	D	1.84±0.190	2.99±0.443	3.40±0.162	**3.48±0.192[b]**

n=10/group

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