

Effect of a hydroethanol 70% extract from trunk bark of *Terminalia superba* Engl. and diels (combretaceae) on some serum biochemical parameters in rats

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Received: 13 April 2017

Accepted: 09 May 2017

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ABSTRACT

Background: In the framework of the valorization of traditional medicine, the biotolerance study of a hydroethanol 70% extract from trunk bark of *Terminalia superba* (HE 70%) Engl. and Diels (Combretaceae), a medicinal plant used for the treatment of gastric ulcer in Côte d'Ivoire was carried out by oral administration repeated for 28 days to three rat groups at doses 250, 500 and 750 mg/kg per body weight (b.w.).

Methods: Fifty rats were randomly divided in four groups of ten animals including three test groups and one control group. Each group included five male and five female rats. An additional satellite group of ten rats in group treated at high dose (750mg/kg b.w.) was included in order to observe reversibility, persistence or late appearance of toxic effects at least 14 days after stopping the treatment. Three doses were prepared (250; 500 and 750mg/kg b.w.) corresponding to extract concentrations (12.5; 25 and 37.5mg/ml) were given to groups B, C and D respectively. Group A, served as control group, received distilled water at 2ml/kg b.w. Groups B, C and D, received orally HE 70% extract at 250, 500 and 750mg/kg respectively. Serum AST, ALT, total and direct bilirubin, total, HDL, LDL-cholesterols, triglycerides, urea and creatinine were estimated using standard methods.

Results: The blood withdrawal analysis done previously (day 0) and at the end of every week on dry tubes revealed that at all weeks and for all doses, this extract do not affect serum values of total and direct bilirubin, creatinine, total, HDL, LDL-cholesterols and triglycerides. Oppositely, this extract reduced significantly ($P < 0.05$) ALT serum rate at the 14th and 28th day at 750mg/kg b.w. respectively. In addition, at the 28th day, AST rate decreased significantly ($P < 0.05$) at 750mg/kg b.w. Glycemia showed a significant ($P < 0.05$) reduction at the 28th day at doses 500 and 750 mg/kg b.w. In contrast, urea increased significantly ($P < 0.05$) at the 28th day at 500mg/kg b.w.

Conclusions: This study showed that the use of a hydroethanol 70% extract from trunk bark of *T. superba* would be hepatoprotective, nontoxic for kidneys, liver and hypoglycemic at the studied doses.

Keywords: Biochemical parameters, Hydroethanol 70% extract, Rat, *Terminalia superba*

INTRODUCTION

For a long time, plants served as first source of drugs for human who used them as therapeutic remedies.¹ In this case, more than 50 000 therapeutic species were listed.² Medicinal plants constitute the major source of drugs resulting from traditional medicine.³ For this reason, the

World Health Organization (WHO) asserts that people in developing countries who use traditional medicine for their primary health care are estimated at 80% approximately.⁴ It is therefore established that medicinal plants play an important role in the fight against the majority of pathologies and are considered harmless to the organism because they are natural.^{4,5} However, their empirical use exposes populations to intoxications that

can sometimes be tragic.⁶ Some recent studies have shown that 35% of acute renal insufficiency and liver damage are associated with the use of herbal medicines.^{6,7} Considering this enthusiasm without reserve for the traditional medicine and the dangers to which its adepts would expose themselves, WHO recommends evaluating the quality, efficacy and safety of medicinal plants in order to establish the scientific proof of their therapeutic effectiveness and harmlessness.⁴

Accordingly we were interested in *T. superba*, a plant of African pharmacopeia, used for the treatment of much pathology such as gastric ulcer, diarrhoea and hemorrhoids.⁸⁻¹¹ Indeed showed that hydroethanol 70% extract from trunk bark of *T. superba* possessed antiulcer properties by increasing mucus production significantly and reducing gastric acid secretion at doses ranging from 125 to 500mg/kg b.w. According to these same authors, this extract contains phytoconstituants such as polyphenols, tannins, flavonoids, quinones, coumarins, saponins, reducing sugars, proteins, sterols and polyterpenes, responsible for its antiulcer activity.¹²

In Côte d'Ivoire, an epidemiologic study revealed that "Antilaléca", a plant mixture has been mentioned in occurred of the intravascular haemolysis and hepatitis in patients.¹³ A pharmacovigilance report revealed that a hydroalcoholic extract of *Camellia sinensis* marketed in France under the name of Exolise was incriminated in occurred of hepatic cytolysis.⁷

In order to prevent such accidents, organizations as WHO and OECD prescribed research lines on medicinal plants to ensure their safety.^{14,15} Thus, taking into consideration what precedes and seen scientific data absence on the safety of the hydroethanol 70% extract from trunk bark of *T. superba* (HE 70%), it is necessary to study the safety of this extract on the organism which receives it.

METHODS

Plant material

Trunk barks of *T. superba* were collected locally from the forest of Ebilassokro village in the East of Côte d'Ivoire in October 2015. Taxonomical identification of those trunk barks was established by botanist from the National floristic Center of University of Felix Houphouët Boigny, Cocody- Abidjan, Côte d'Ivoire, 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.

Preparation of a hydroethanol 70% extract from the trunk bark of Terminalia superba

Extraction is based on *T. superba* traditional method preparation. Trunk barks of *T. superba* were dried under shade and powdered with a machine (mark RETSCH, type SM 100, Germany). The extraction process was

implemented according to the method described by.¹⁶ One hundred grams (100g) of the trunk barks powder were macerated during 24 hours in 11 ethanol-water (70:30 v/v) for 3 times until complete exhaustion. The mixtures were filtered (Whatman n°1) and concentrated under reduce pressure using a rotary evaporator (Büchi R110, type MKE 6540/2) at a temperature of 45°C. The concentrated extracts were stored in dessicators at 45°C.

Animals

Albino wistar rats of either sex weighing between 103 and 114 g. Selected animals for this study were aged from three to eight weeks. They were bred in Animal house of Physiology, Pharmacology and Pharmacopeia laboratory of the University of Nangui Abrogoua (Abidjan, Côte d'Ivoire) according to the principles for the care and use of laboratory animals of the Ethical Committee of the University (Nangui Abrogoua, Abidjan, Côte d'Ivoire). They were exposed to 12 hours dark/light cycle.

Chemical substances

The chemicals used are: Ether (VWR International-Geldenaakfebaan 464-B-3001, Leuven-Belgium), 96° ethanol (Carlo Erba Reagents) and distilled water.

Subacute toxicity study

It was carried out according to OECD Guidelines 407 which consists in daily administering Hydroethanol 70% extract from trunk bark of *T. superba* (HE 70%), increasing doses to four groups of rats at one dose per groups for 28 days orally.¹⁷

Fifty rats were randomly divided in four groups of ten animals including three test group and control group. Each group included five male and five female rats. An additional satellite group of ten rats in group treated at high dose (750mg/kg b.w.) was included in order to observe reversibility, persistence or late appearance of toxic effects at least 14 days after stopping treatment. Three doses were prepared according to.¹² Doses 250; 500 and 750mg/kg b.w. corresponding to extract concentrations (12.5; 25 and 37.5mg/ml) were given to groups B, C and D respectively. Group A, served as control group, received distilled water at 2ml/kg b.w. Groups B, C and D, received orally HE 70% extract at 250, 500 and 750mg/kg respectively. Before administering various treatments, animals of each group were marked and weighed individually.

Blood withdrawals were done in each rat at the beginning of the experiment (Day 0) and at the end of each week until the end of the experiment (Day 28) for biochemical analysis.

Thus, at the day 0 and the end of each week, the fasted animals, the day before evening, were anesthetized with

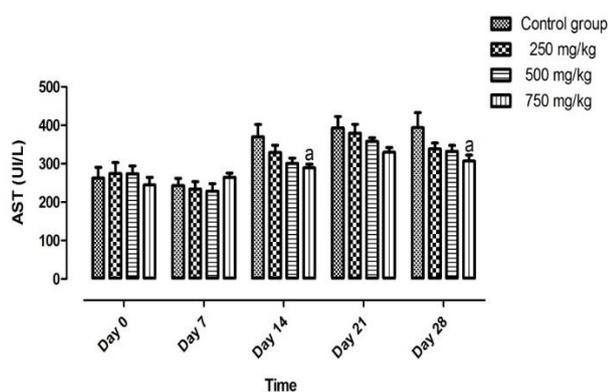
ether and blood was collected early in the morning by ocular puncture in dry centrifuged tubes containing an anticoagulant (EDTA) and was centrifuged at 3000 rpm for 5 minutes to get preserved serum in the cups at - 20 ° C for the estimation of biochemical parameters, namely, urea, triglycerides and total cholesterol by enzymatic method; creatinine by colorimetric method, transaminases (ALT and AST) by kinetic method, bilirubins by Diazo method using a semi Robonic® automaton TOUCH prietest.¹⁸⁻²³ Glucose was carried out on whole blood at withdrawal time using Accu-chek® glucose meter according to glucose oxidase method.²⁴

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 5.01 (San Diego, California, USA) software. All values were expressed as mean±standard error of the mean (m±s.e.m).The average comparisons was made compared to control, because of ANOVA repeated measurements with mixed model to check variable normality. The significance of the differences observed between the average values of the various parameters was implemented by analysis of variances (ANOVA) with posthoc Bonferroni multiple comparison testing. P values less than 0.05 were considered significant.

RESULTS

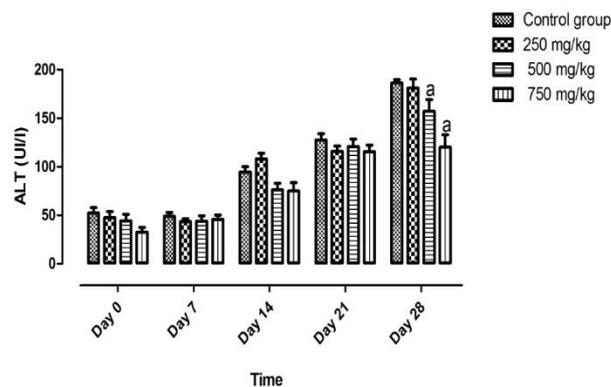
Oral administration of HE 70 % extract in repeated doses (250, 500 and 750 mg/kg b.w.) to rats for 28 days produced significant (p <0.05) decrease in ALT serum rate at 750 mg/kg on the 14th and the 28th day of treatment (Figure 1).



n= 10 rats in each group; a p <0.05: significant decrease compared to control group

Figure 1: Effect of a hydroethanol 70% extract from trunk bark of Terminalia superba on AST serum rate for 28 days in rat.

As for AST serum rate, a significant decrease (p <0.05) at 500 and 750mg/kg b.w. was observed compared to control group at the 28th day of treatment. This decrease is highly significant (p <0.001) compared to that recorded at 500 mg/kg b.w. on the 28th day of treatment (Figure 2).



n= 10 rats in each group; a p <0.05: significant decrease compared to control group

Figure 2: Effect of a hydroethanol extract from trunk bark of Terminalia superba on ALT serum rate for 28 days in rat.

In addition, animal treated for 28 days with repeated doses of HE 70% extract does not significantly (p >0.05) alter total and direct bilirubin serum rates (Table 1). Moreover, no significant change (p >0.05) between creatinine average values of treated groups at 250; 500 and 750mg/kg respectively and those of the control group (Table 1) was observed following HE 70% extract administration in repeated doses during various weeks.

However, urea serum rate, underwent a significant (p<0.05) increase at 500 mg/kg on the 28th day of treatment (Figure 3).

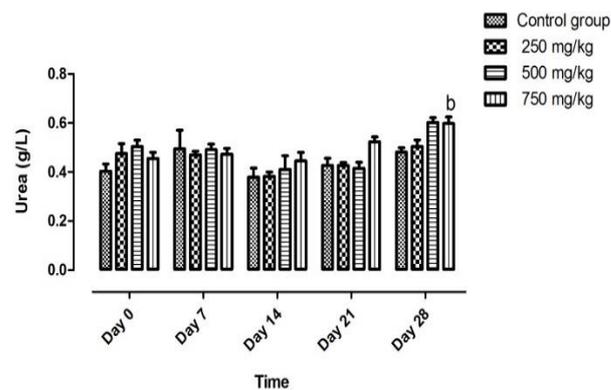


Figure 3: Effect of a hydroethanol extract from trunk bark of Terminalia superba on urea serum rate for 28 days in rat. n= 10 rats in each group; bp <0.05: significant increase compared to control group.

Triglycerides, total, HDL and LDL-cholesterol rates were not disrupted (p >0.05) during the 28 days of treatment (Table 2) contrary to glycaemia which underwent on the 28th day, a significant (p<0.05) decrease at doses 500 and 750 mg/kg (Figure 4). The values in the same column are not statistically different from the control at p <0.05); n = 10 rats in each group.

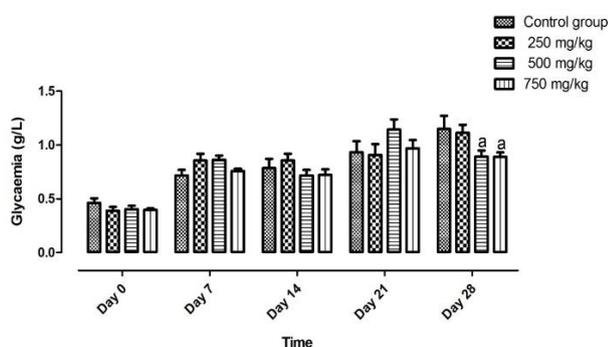
Table 1: Evolution of liver and kidney serum biochemical rates of rats treated with a hydroethanol 70% extract for 28 days.

Biochemical parameters	Doses (mg/kg b.w.)	Day 0	Day 7	Day 14	Day 21	Day 28
Total bilirubin (mg/l)	Control	0.3939±0.0459	0.5098±0.0906	0.5743±0.0380	0.4797±0.0139	0.3610±0.0668
	250	0.3770±0.0494	0.5190±0.0660	0.5830±0.0539	0.5127±0.0097	0.3960±0.0691
	500	0.3340±0.0648	0.5485±0.1235	0.5871±0.0962	0.5041±0.0144	0.5420±0.0964
	750	0.3620±0.0361	0.5827±0.0883	0.5697±0.0976	0.5381±0.0124	0.5520±0.0840
Direct bilirubin (mg/l)	Control	0.4390±0.0602	0.5328±0.0523	0.5902±0.1372	0.5390±0.0755	0.5180±0.0994
	250	0.4360±0.0272	0.5025±0.0740	0.5847±0.0330	0.4958±0.0688	0.4920±0.0751
	500	0.4260±0.0695	0.4733±0.0796	0.5215±0.0781	0.4364±0.0883	0.4480±0.0480
	750	0.4340±0.0432	0.4272±0.0696	0.5097±0.0299	0.4456±0.0630	0.3470±0.0841
Creatinine (g/l)	Control	0.8304±0.0490	0.5211±0.0389	0.6311±0.0303	0.5974±0.0360	0.6286±0.0964
	250	0.7575±0.0429	0.5347±0.0200	0.5419±0.0199	0.5414±0.0167	0.5607±0.0629
	500	0.7164±0.0844	0.4974±0.0442	0.5673±0.0509	0.4463±0.0216	0.5045±0.0694
	750	0.7202±0.0784	0.4903±0.0522	0.0504±0.0440	0.4415±0.0402	0.5013±0.0380

The values in the same column are not statistically different from the control at $p < 0.05$; $n = 10$ rats in each group

Table 2: Evolution of the lipidic profile of rats treated with a hydroethanol 70% extract for 28 days.

Biochemical parameters	Doses (mg/kg b.w.)	Day 0	Day 7	Day 14	Day 21	Day 28
Triglycerides (g/l)	Control	1.7430±0.1127	1.4930±0.1861	1.0970±0.1936	1.5860±0.1039	1.1170±0.0854
	250	1.7370±0.1793	1.2500±0.1563	1.265±0.0702	1.5910±0.0535	1.0570±0.0997
	500	1.7340±0.3342	1.1760±0.1493	1.0370±0.1072	1.4900±0.0520	1.0700±0.0821
	750	1.9050±0.2192	1.3550±0.2141	0.9928±0.1402	1.4650±0.9366	1.0910±0.1082
Total Cholesterol (g/l)	Control	1.0810±0.0628	1.1440±0.0548	1.1710±0.0395	1.1750±0.0720	1.1500±0.0325
	250	1.1140±0.0545	1.0450±0.0227	1.1580±0.0298	1.0250±0.0569	1.0610±0.0446
	500	1.1580±0.0574	1.1030±0.0279	1.1680±0.0374	1.1210±0.0136	1.0420±0.0779
	750	1.0240±0.1085	1.1250±0.0398	1.1370±0.0320	1.1030±0.0174	1.0590±0.0890
HDL Cholesterol (g/l)	Control	0.6424±0.682	0.4899±0.0983	0.7252±0.0351	0.6237±0.0621	0.6514±0.0454
	250	0.6189±0.0618	0.5511±0.0577	0.5603±0.0644	0.7758±0.0338	0.7738±0.0361
	500	0.6403±0.0753	0.5997±0.0500	0.9121±0.1016	0.6659±0.0630	0.7567±0.0341
	750	0.5074±0.0574	0.5819±0.0068	0.7561±0.0392	0.6392±0.0380	0.7789±0.0335
LDL Cholesterol (g/l)	Control	0.2385±0.0712	0.4641±0.1118	0.2630±0.0639	0.3181±0.0639	0.2755±0.0434
	250	0.2341±0.0573	0.2761±0.0534	0.3772±0.0669	0.1066±0.0369	0.2101±0.0530
	500	0.3075±0.1123	0.2677±0.0491	0.3010±0.0544	0.3169±0.0920	0.1829±0.0449
	750	0.3488±0.0615	0.2878±0.0402	0.2190±0.0496	0.1846±0.0242	0.2324±0.0398



$n = 10$ rats in each group; a $p < 0.05$: significant decrease compared to control group

Figure 4: Effect of a hydroethanol extract 70% from trunk bark of *Terminalia superba* on glycaemia serum rate for 28 days in rat.

DISCUSSION

Oral administration of HE 70% extract from trunk bark of *T. superba* in repeated doses (250, 500 and 750mg/kg b.w.) for 28 days to rats, revealed that this extract significantly reduced transaminase serum levels (AST and ALT). These liver biomarkers (AST and ALT) are serum enzymes which have significant metabolic activities inside the cells. They were synthesized in cell cytoplasm and were discharged in circulation when cells were damaged.²⁵ In this case, they were said to be good hepatic cytolysis indicators. When these liver biomarkers blood rate was low, that indicated liver good functional level.²⁶ Oppositely, increase in their rate indicated a cellular lesion especially in liver, heart, kidney or muscle.^{27,28} The decrease in AST and ALT serum rates in treated animals at the three doses of HE 70% extract for

28 days would represent, liver and heart protection and any muscular lesion. These results were similar to those of.²⁹ Indeed, these authors observed a decrease in these two enzymes serum rate in rats treated with methanolic extract of the stem bark of *Crossopteryx febrifuga* at 1000mg/kg b.w. According to, the total aqueous stem bark extract of *Spondias mombin* did not cause disturbances in myocardial and skeletal muscle cells of rats.²⁹

In addition, total and direct bilirubin were not disrupted during this study indicating that liver did not undergo any injury during the treatment with these different doses of HE 70% extract. However it is well-known that bilirubin is from the conversion of the heme released from the erythrocytes.³⁰ It may be in free or conjugated form. High bilirubin serum rates were due either to inability of the kidneys to remove excess bilirubin or to a failure of the liver to transform bilirubin before its excretion.³¹ Thus, the ability of the hepatoprotective substances to reduce harmful effects or to preserve liver functioning mechanisms against hepatotoxin disturbances was an indication of their protective effect.³² The decrease in total and direct bilirubin rates by HE 70% extract of *T. superba* was the proof of its protective effect and effectiveness on hepatic cells. These results corroborated those of that showed that oral administration of ethanolic extract of *Piliostigma reticulatum* did not change total and direct bilirubin serum rates.³³

HE 70% extract of *T. superba* (250; 500 and 750mg/kg b.w.) administered repeatedly for 28 days did not alter creatinine serum rate. This result testifies a normal performance of kidneys. This is an indication that the extract would be without effect on renal function. In contrast, HE 70% extract of *T. superba* significantly increased urea serum rate on the 4th week of the treatment at 750mg/kg. The increase in urea serum rate in rats treated with the three doses of the HE 70% extract would be explained by the degradation of the proteinic compounds brought by food which can be transformed into amino acids and then into urea. These results expressed an activation of ureogenesis which was a liver specific function.³⁴ Urea was the ultimate step in the catabolism of free amino acids. These results were in opposition to those obtained by that showed that the total aqueous extract of the trunk bark of *T. superba* significantly reduced urea rate at doses of 250 and 500mg/kg b.w. on the 4th week.³⁵

Triglycerides, HDL and LDL-cholesterols of the rats treated during 28 days showed clearly that HE 70% extract of *T. superba* did not disturb these blood parameters. Indeed, it is well-known that alterations in the concentrations of major lipids like cholesterols and triglycerides can give useful information on the lipid metabolism as well as predisposition of the animals to atherosclerosis and cardiovascular complications.³⁶ Increasing in serum rate of these lipids except cholesterol-HDL was associated with the greatest risk of

atherosclerosis. Thus, the absence of any effect of HE 70% extract on all the lipidic parameters investigated, suggested that the lipid metabolism in the animals was not altered. This could be an indication that HE 70% extract could not probably predispose animals to atherosclerosis and cardiovascular diseases. These results are similar to those of who observed that administration of an aqueous leaves extract of *Parkia biglobosa* (Mimosaceae) in rabbits, did not alter lipidic parameters at 500, 1000 and 1500 mg/kg b.w.³⁷

In addition, repeated doses administration of HE 70% extract of *T. superba* (250, 500 and 750mg/kg b.w.) for 28 days induced significant decrease of the glycemia rate at 500 and 750mg/kg b.w. on the 4th week. These results that indicated the hypoglycemic effect of the extract were contrary to those obtained by that showed that total aqueous extract of the stem bark of *Spondias mombin* did not have any effect on rats' glycemia. However, further additional experiments are necessary to confirm it.²⁹

CONCLUSION

This study showed that a hydroethanol 70% extract from trunk bark of *T. superba* administered orally to rats in the period of 28 days, reduced considerably transaminases (AST and ALT) serum rates. It did not disturb total, direct bilirubin and creatinine serum rates. This extract increased urea serum rate and was without effect on lipid metabolism. It would be hepatoprotective, nontoxic for kidneys, liver and hypoglycemic at the studied doses and may justify the traditional use of the plant in ulcer treatment. Therefore, histopathological investigations are needed to confirm or refute these results.

ACKNOWLEDGMENTS

Authors would like to thankful to all other members of Laboratory of Physiology, Pharmacology and Pharmacopoeia for their encouragement during these investigations.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Bernard GN, Mathieu NN, Paul YA. Effect of a hydroethanol 70% extract from trunk bark of *Terminalia superba* Engl. and diels (combretaceae) on some serum biochemical parameters in rats. *Int J Basic Clin Pharmacol* 2017;6:1242-8.