Title : Evaluation of the toxicity of the ethanol extract70% of the bark of Terminalia macroptera (Combretaceae) on whistar rats.

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ABSTRACT

Nowadays, many medicinal plants have proved effective in combating the phenomenon of bacterial multi-resistance against conventional antibiotics. However, the use of these plants, traditionally is done without precise doses. And this inaccuracy of dose is a real problem of traditional medicine. Thus prospecting for empirically administered plant extract requires dosage monitoring to avoid the risk of a fatal therapeutic accident. It is in this context that the study of the toxicity of *Terminalia macroptera* which presents itself as an anti-infectious agent, capable of overcoming certain strains of antibiotic-resistant bacteria has been initiated. The objective of this study is to evaluate the toxicity of 70% ethanol extract of T.macroptera in rats and to deduce its safety. With regard to the evaluation of the toxicity, rats were used whose mass varies between 100 and 170 grams. Then, using OECD Guideline 425, (2006), acute toxicity was achieved. Then the 100, 300 and 500 mg / kg bm doses were used in sub-acute toxicity to evaluate biochemical and hematological parameters. The results show an LD_{50}> 5000 mg / kg bm. Therefore, according to the OECD classification, the hydroethanolic extract belongs to category 5, non-toxic substances. Also, the biochemical and hematological results revealed that the extract did not change at any time at P <0.05, biochemical marker levels (UREE, ASAT, ALAT, CK and LDH), reflecting vital organs of the body. So the extract would have no effect on the heart, liver and kidneys. 70% ethanol

extract of T.macroptera would be safe for use as a drug and therefore could contribute to the production of Traditionally Enhanced Medicines (MTAs).
Keywords: Terminalia macroptera – Toxicité - Triphytochimie

Introduction

Today, infectious diseases account for nearly 17 million deaths a year, accounting for one-third of global mortality**[1]**. They constitute 45% of deaths in developing countries, including 14.2% in Côte d'Ivoire **[2]**. Thus, this morbidity is likely to worsen, given the climatic changes caused by the industrialization of modern societies, which therefore create and favor an ideal temperature for the proliferation and spread of pathogens. The treatment of these infections has become increasingly difficult because of the emergence of multi-resistant strains **[3]**, aimed at antibiotics usually used Thus plant flora becomes a therapeutic alternative for the scientific world because, more than 80% of African populations use medicine and traditional pharmacopoeia to cope with their health problem **[4,5]**. This is the case of studies conducted by many researchers on medicinal plants used in our villages. However, the traditional medical art, despite these advantages, faces many obstacles, such as the doses used in the preparation and administration of phytomedicines **[6]**. Traditionally, the doses to be administered are expressed in different ways: a few drops of the product in the form of instillation auricular, oral, nasal, ocular, vaginal, a cup, a ladle, a shot glass, a cup, a teaspoon, a tablespoon or a handle**[7]**. The doses used remain imprecise**[8]**. This inaccuracy is a real problem of traditional medicine. Prospecting for empirically administered extracts therefore requires dose monitoring to avoid the real risks of a therapeutic accident that can sometimes be tragic **[9].**It is in this context that we initiated this study on *Terminalia macroptera*, which is presented as an anti-infectious capable of overcoming certain strains of bacteria, and even the most resistant at present.

Matériels et méthodes

Plant Material It consists of bark *Terminalia macroptera* Guill. and Perr. (*Combretaceae*). These barks were collected in April 2016 in Niakara (north of Ivory Cost). The authentication was performed by professor Ake-Assi, Director of National Floristic Center (NFC),University Felix Houphouet Boigny of Cocody-Abidjan. In this Center a sample is kepted..

Matériel végétal

Preparation of plants extracts :

The stem barks of *T. macroptera*are collected, washed, cut and has been dried shelter powder by a type IKAMAGRCT grinder. According to the methods described by Bagré **[10]**, 100 g of plant powder have been macerated in 1 L of distilled water then homogenized under magnetic agitation during 24 hours at 25° C with a IKAMAG-RCT type agitator. The homogenate obtained, has been filtered successively two times through hydrophilic cotton (cotton wool) then once through whattman paper n°2. The volume of filtrate obtained is first reduced with a rot vapor Büchi at 60° C. Then, the rest of the filtrate is evaporated with a

med center vent cell,anddrying oven at 50° C to give a brown powder, which is the aqueous extract. The same process was carried out by using ethanol 70% instead of distilled water, to obtain ethanolic extract 70% **[11]**. At The end, all the plant extracts obtained are kept in refrigerator until used for testing.

Animal material

Male and female whistar rats ranging in age from 8 to 12 weeks and body mass ranging from 100 to 190 g were used from the Pasteur Institute Animal Resource Management Unit (UGRA). From Ivory Coast.

Méthodes

Phytochemical analysis

The phytochemical analysis of the different extract of *Terminalia macroptera* have based on the coloration and precipitation test **[12, 13].**

Test for alkaloids : 0.5 g of extract was diluted into 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggen dorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggen dorff's reagent) was regarded as positive for the presence of alkaloids.

Test for polyphenolsand tannins : 0.5 g of the extract was boiled into 10 ml of water in a test tube and then filtered. A few drops of 0.1% of ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for terpenoids : (Salkowski test) To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H2SO4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for glycosides : Extracts was treated with 2 ml of glacial acetic acid, add 1 drop of FeCl3 and 1 ml of concentrated H2SO4 appearance of brown coloration indicates the glycosides.

Test for flavonoids : Three methods were used to test for flavonoids. First, dilute ammonia (5ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1ml) was then added. A yellow coloration that disappears on standing indicates the presence of flavonoids. Secondly, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow coloration indicates the presence of flavonoids. Next, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for saponins : To 0.5 g of extract was added 5 ml of distilled water in test tube. The solution was shaken and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken after which it was observed for the formation of an emulsion.

Fehling's test: Filtrates were hydrolysed with dil. HCL neutralized with alkali and heated with fehling's A and B solution. Formation of red precipitate indicates the presence of reducing sugars.

Test for steroids and terpenoids: 9 ml of ethanol was added to 1 g each of the extracts and refluxed for a few minute and filtered. Each of the filtrates was concentrated to 2.5 ml in a boiling water bath. Distilled water, 5ml was added to each of the concentrated solution, each of the mixtures was allowed to stand for 1 h and the waxy matter was filtered off. Each of the filtrates was extracted with 2.5 ml of chloroform using a separating funnel. To each 0.5 ml of the chloroform extracts in a test tube was carefully added 1 ml of concentrated sulphuric acid to form a lower layer. A reddish-brown interface showed the presence of steroids. To another 0.5 ml each of the chloroform extract was evaporated to dryness on a water bath and heated with 3 ml of concentrated sulphuric acid for 10 min on a water bath. A grey color indicates the presence of terpenoids.

Study of the toxicity of the ethanol extract 70% of *T. macroptera*

1. Acute toxicity study

The acute toxicity was used to evaluate the toxic effects that occur within a short time (1 to 14 days) after administration of a single dose substance.
The desired effects include:
-The abdominal contraction
-The abnormal gait
-The reduced activity
-The breathing: change of rhythm, convulsion
Diarrhea
-Letality or mortality

Experienced animals

Whistar breed rats, fed with good health, female and male ranging from 100 to 190 grams. Rats, eight to twelve weeks old, were kept in clean cages at a rate of 6 rats per cage in a well-ventilated room. Rats were acclimated to experimental conditions.

Preparation of different concentrations of the ethanol extract of *T.macroptera* to be administered to the animals.

The different concentrations of the 70% ethanolic extract were prepared taking into account the body weight (mc) of the rats and the amount of product to be injected. It is expressed in mg / kg of body weight (mg / kg / mc). The animals having for example a mass of 160 grams for a maximum dose (DM) of 5000 mg / kg of body weight, the amount (q) of the total ethanolic extract 70% of *T. macroptera* to be administered was given by the next calculation called "the rule of three".

1000 g → 5000 mg
160 g → q?
qx1000 = 5000x160

q = (5000x160) / 1000
Q = 80 so q = 80 mg of extract per rat.
Since this quantity is administered in volume of 2 ml to the rat, the corresponding concentration is given by:
C = q / v C = 80/2 = 40 mg / mL
Thus the different concentrations administered corresponding to the doses are recorded in **Table I.**

Oral administration

Rats previously fasted for 24 h are grouped into 3 lots of 6 rats each. Either 18 rats to administer. The following doses were administered by gavage:
In batch 1 (Control), the rats received only distilled water at a rate of 2 ml per rat.
In batch 2, the rats received the maximum dose of 2000 mg / kg mc at a rate of 2 ml per rat.
In batch 3, the rats received the maximum dose of 5000 mg / kg mc at a rate of 2 ml per rat.
After administration the observation was made for 2 hours before feeding.

Clinical observation

Signs of toxicity and mortality were noted for each animal. After administration of the extract, the animals were observed individually and regularly during the first 30 minutes during the first 24 hours with particular attention during the first 4 hours. Subsequently, observations were made daily over a period of 14 days. During this observation period, the number of deaths as well as the symptomatic disorders were noted.

Table 1: Doses Administered During Acute Toxicity

Parameters	Doses (mg/Kg/mc		
	Temoins	2000	5000
Numbers	6	6	6
Average weight	160	160	160
Extract (mg)	0	32	80
Concentration (mg/mL)	0	16	40
Volume administered (mL)	2	2	2

2. Sub-acute toxicity study.

It has been determined from OECD Guideline 407 **[14]** . The rats aged 8 to 12 weeks were divided into 4 batches in cages of 6 rats each, ie 24 rats, for 28 days.

Preparation of different concentrations of extract

The different concentrations of the 70% ethanolic extract were prepared taking into account the body mass (mc) of the rats and the amount of product to be injected. It is expressed in mg / kg of body weight (mg / kg / mc). The animals having for example a mass of 157.32 g for a dose of 500 mg / kg of body weight, the amount (q) of the total ethanolic extract 70% of *T. macroptera* to be administered was given by the following calculation. :

500 mg → 1000 g
157.32g → q?
qx1000 = 500x157, 32
q = (500x157.32) / 1000
q = 78.66 so q = 80 mg of extract per rat.

Since this quantity is administered in volume of 2 ml to the rat, the corresponding concentration is given by:
C = q / v C = 78.66 / 2 = 39.33 mg / mL
Thus the different concentrations administered, corresponding to the doses are recorded in

Table 2: Doses Administered duringSub-acute toxicity

Paramètres	Doses (mg/Kg/mc)			
	Control	100	300	500
Nombers	6	6	6	6
Average weight (g)	112.60	116.20	138.70	157.32
Extract(mg))	0	11.62	41.61	78.66
Concentration(mg/mL)	0	5.81	20.80	39.33
Volume administered	0	2	2	2

Oral administration

Rats previously fasted for 24 h are grouped into 4 lots of 6. Either 24 rats to be administered. The dose of 500 mg / kg bw was obtained using 1/10 DMT of acute toxicity and then a low dose of 100 mg / kg bw equivalent to 1/5 of 500 mg / kg bw and an intermediate dose of 300 mg / kg bw greater than or equal to half the dose of 500 mg / kg bw. Thus the following doses were administered by gavage:
In batch 1 (Control), the rats received only distilled water at a rate of 2 ml per rat.
In batch 2, the rats received the dose of 100 mg / kg body weight at a rate of 2 ml per rat.
In batch 3, the rats received the dose of 300 mg / kg body weight at a rate of 2 ml per rat.
In Lot 4, the rats received the dose of 500 mg / kg of body weight at a rate of 2 ml per rat.
After administration the observation was made for 2 hours before feeding the rats.

Clinical observation

Clinical signs of toxicity were observed daily in all rats before, immediately, and 3 hours after administration of the ethanolic extract solution

Variation in body weight of rats during 28 days of feeding.

The rats before each extract administration were weighed using a scale (Sartorius / BP 2215) starting from the first to the 28th day. The average of the mass was then calculated according to each batch of dose (mg / kg of mc) and per day. Thus the change in body mass was determined by the average mass difference between the first and the 28th day.

Blood sample

At the 29th day or the day after the last day of the treatment, the animals were anesthetized with ether and the blood of the rats was taken from the tail by amputation of the tip of the tail (5 mm of the tip) previously disinfected with ethanol 96 ° C then distributed in a dry tube and a tube containing anticoagulant ethylene diamine tetra-acetite acid (EDTA).

Biochemical analysis

Assay of Aspartate Aminotransferase (ASAT)

Aspartate aminotransferase (ASAT) was assayed according to the Karmen / Bergmeyer method of coupling of malate dehydrogenase and nicotinamide reduced nucleotide (NADH), described by **[15]**.

ASAT transfers its amino group to the carbon atom of α-keto glutarate with formation of glutamate and oxaloacetate. The oxaloacetate is then reduced to malate by malate dehydrogenase in the presence of reduced NADH which oxidizes to NAD^+. The amount of NADH reduced is proportional to the intensity of the oxaloacetate present in the serum and therefore to the intensity of ASAT activity. This catalytic activity is obtained by determining the disappearance of NADH at 340 nm. The reaction is as follows:

Equation 1:

L-Aspartate + α – Cetoglutarate $\rightleftharpoons$ Oxaloacetate + L-Glutamate

Oxaloacetate + NADH + H $\rightarrow$ L-malate + NAD^+ (1)

The dosage was done as follows

In a heparinized EDTA tube containing 500 µl of reagent (total volume) pre-incubated at 37 ° for 2 to 3 min, 50 µl of the sample to be assayed are added. After stirring for 1 min, the optical densities were read spectrophotometer at 350 nm to determine the serum enzyme activity of ASAT. This activity is calculated from the SGOT factor (F_SGOT = 1745) and that of λ which is 340nm according to the formula below.

Equation 2: Enzyme activity of SGOT

$$\text{The enzymatic activity of SGOT (UI)} = (\Delta DO) / \lambda \times 1745 \quad (2)$$

Determination of Alanine Aminotransferase (ALAT)

Alanine aminotransferase (ALAT) was measured according to the method recommended by the International Federation of Clinical Chemistry (FICC), described by **[15]**.

The principle of the reaction is the following; Alanine aminotransferase (ALAT) catalyzes the transfer of the amine group from alanine to α-ketoglutarate to form pyruvate and L-glutamate. Pyruvate is converted to lactate by lactate dehydrogenase as shown in the following equation:

Equation 3: Pyruvate conversion equation

$$\text{L-Alanine} + \alpha\text{-ketoglutarate} \xrightleftharpoons{\text{ALAT}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H} + \text{L-Lactate} + \text{NAD} + \xrightarrow{\text{LDH}} \quad (3)$$

The rate of reduction of NADH is proportional to the amount of pyruvate formed in the medium and therefore to the activity of alanine. This activity is determined by measuring the absorbance at 350 nm against a control.

The dosage was as follows

In a heparinized EDTA tube containing 500 µL of reagent (total volume) preincubated at 37 ° for 2 to 3 min, 50 µL of the sample to be assayed was added. After stirring for 1 min, the optical densities were read on a spectrophotometer at the wavelength λ equal to 350 nm to determine the serum enzyme activity of ALAT. This activity is calculated from the SGOT factor (F_SGPT = 1745) and that of λ which is 350nm according to

Equation 4:

$$\text{The enzymatic activity of SGOT (UI)} = (\Delta DO) / \lambda \times 1745 \quad (4)$$

Determination of creatinine

The creatinine assay was performed according to the Jaffé method **[16]** and improved by more specific enzymatic methods for creatinine **[17, 18],** to increase the specificity of the reaction.

In alkaline medium, creatinine forms with sodium picrate an orange-red complex which absorbs at 500 nm. The increase in absorbance, which corresponds to the rate of development of the staining, is proportional to the concentration of creatinine in the medium. Thus, in a series of three tubes (standard, blank and assays) each containing 500 µl of the reaction medium, 100 µl of creatinine standard, 100 µl of distilled water and 100 µl of sample were respectively added. The tubes are shaken, and after 30 seconds a first absorbance A1 of each tube is read at 500 nm against a control tube. 90 seconds after the first reading a second reading of the absorbance A2 was made at 500 nm on the spectrophotometer. The creatinine concentration was calculated according to

Equation 5:

$$(A_2\text{-}A_1)\ \text{Sample}$$

Concentration of creatinine in mg $\longrightarrow$ X standard concentration

$(A_2\text{-}A_1)$ Standard (5)

Determination of urea

Urea was determined according to the Berthelot method described by [15]. Urea is hydrolysed in the presence of water and urease to produce ammonia and carbon dioxide. Ammonia, in the presence of glutamate dehydrogenase (GLDH) and nicotinamide adenine dinucleotide in reduced form (NADH), combines with acetoglutarate (α-CG) to form L-glutamate and a blue-green colored complex. The decrease in the concentration of NADH in the medium which is observed by the decrease in the absorbance of the 340 nm stain is proportional to the urea concentration of the sample as indicated in

Equation 6. Urease

Urea + H2O $\longrightarrow$ NH3 + CO2

$$\text{NH3} + \alpha\text{-CG} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-Glutamate} + \text{NAD}^+ \quad (6)$$

Thus, in a series of three tubes (standard, blank, tests) each containing 1000 µl of reaction medium, 100 µl of urea standard, 100 µl of distilled water and 100 µl of sample were respectively added. After stirring automatically for 1 min and incubating at room temperature (16-25 ° C), the optical densities of each tube were read spectrophotometrically at 600 nm to determine the serum enzyme activity of the urea. This activity is calculated

Equation 7:

$$\text{Urea concentration in g /L} = \frac{\text{DO Sample}}{\text{DO Standard}} \times \text{Concentration Standard} \quad (7)$$

Determination of creatinine kinase (C.K)

Creatinine Kinase (CK) was assayed according to the method of Picololo as modified by the International Federation of Clinical Chemistry, described by [19].

The method of assaying CK is based on the reaction chain as indicated in

Equation 8:

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \longrightarrow \text{Glucose 6-p} + \text{ADP}$$

$$\text{Glucose-6-p} + \text{NADP} \longrightarrow \text{Gluconate 6-p} + \text{NADPH} + \text{H}^+ \quad (8)$$

Thus, in an EDTA tube containing 1000 µL of reagent preincubated at 37 ° C. for 2 to 3 min, 50 µL of the sample to be assayed was added. After stirring for 1 min and incubation for 2 min, the optical densities were read spectrophotometer at 340 nm to determine the CK

enzyme activity from factor CK, F_CK8096 and the wavelength λ equal to 340 nm.Expression of enzymatic activity is given by,

Equation 9:

The enzymatic activity of CK in UI = (ΔX) / λ X 8095 (9)

Determination of total cholesterol

Blood cholesterol comes in two forms namely the free form and the ester form. The esterified form is hydrolysed by cholesterol esterase to give the free form and fatty acids. In the presence of cholesterol oxidase, free cholesterol oxidizes to form 4-cholestene-3-one and hydrogen peroxide. Hydrogen peroxide reacts with hydroxybenzoic acid (HBA) and 4-aminoantipyrine to form red quinoneimine. The absorbance of this color measured at 500 nm is directly proportional to the amount of cholesterol contained in the serum **[20]**. The reaction is given by

Equation 10

$$\text{Esterified Cholesterol} + H_2O \xrightarrow{\text{Cholestérol Esterase}} \text{Cholesterol} + \text{Fatty Acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholestérol Oxydase}} \text{Cholestene-4-one-3} + H_2O_2$$

$$H_2O_2 + \text{Hydroxybenzoic acid} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxydase}} \text{Quinoneimine} \quad (10)$$

Thus, in an EDTA tube containing 1000 µl of reagent preincubated at 37 ° for 2 to 3 min, 10 µl of the sample to be assayed were added. After stirring for 1 min and automatic incubation at room temperature (16-25 ° C) for 10 min; the optical density is read spectrophotometer at 500 nm to determine the concentration of cholesterol according

Equation 11:

$$\text{Cholesterol concentration in g / L} = \frac{DO\ Sample}{DO\ Standard} \times \text{Standard concentration} \quad (11)$$

Determination of blood sugar

The assay method used is the enzymatic method **[21].** It consists in oxidizing glucose by the enzyme glucose oxidase with production of gluconic diacid and hydrogen peroxide (H_2O_2). The peroxide reacts with phenol and 4-amino-antipyrine in the presence of peroxidase to form a brick-red compound, quinone imine and water. The optical density of quinoneimine at 500 nm is proportional to the glucose concentration present in the sample. The principle is summarized in to

Equation 12

$$\text{Glucose} + O_2 + H_20 \xrightarrow{\text{Glucose Oxydase}} \text{Gluconic acid} + H_2O_2$$

Peroxydase

H_2O_2 + Phenol Complex red + 4-aminoantipyric + $4H_2O_2$

(12)

For the assay the blood of the animals was centrifuged at 3500 rpm for 10 min and then the serum collected. 10 microliters of serum was reacted with one milliliter (1mL) of enzymatic solution incubated in a 37 ° C water bath. The reading was then carried out using a KENZA-type spectrophotometer at 500 nm against a blank composed of physiological NaCl 9 solution.

Determination of high density lipoprotein (HDL)

HDL cholesterol (HDL) is a serum marker for the diagnosis of coronary artery disease. Decreasing its concentration in the blood increases the risk of developing the disease. HDL was assayed using the HDL VITROS analytical plaque method based on precipitation of low density lipoprotein (LDL) and VLDL followed by enzymatic detection **[22]**. The VITROS analytical plate is composed of: a spreading layer and underlying layers. The plating layer contains phosphotungstic acid (PTA), magnesium chloride ($MgCl_2$) and Emulgen B-66 surfactant.

For the assay, a drop of the sample is deposited on the plate and uniformly spread in the underlying layers by the spreading layer. Phosphotungstic acid (PTA) and MgCl 2 from the spreading layer are used to isolate HDL by precipitation of LDL and VLDL. The cholesterol esters of the LDH complexes are selectively separated from free cholesterol by the catalytic action of the Emulgen B-66 surfactant. HDL-derived cholesterol esters are hydrolyzed to free cholesterol by the action of a cholesterol ester hydrolase. Free cholesterol is then hydrolysed to cholesterone and hydrogen peroxide. The latter oxidizes a leuco derivative in the presence of peroxidase to form a colored complex whose staining density is proportional to the concentration of HDL-C present in the sample. This density is measured by reflectance spectrophotometry. The assay principle is summarized in the reaction sequence:

Equation 13.

Emulgene B-66

HDL + LDL LDL + VLDL + HDL

Cholesterol-Esters hygrolase

HDL Complex Free Cholesterol + Cholesterol Esters

Cholesterol Ester + H_2 O Free Cholesterol + H_2O_2

Peroxdase

Free cholesterol + H_2 O cholesterone + H_2O_2

H_2O_2+Leucodériue Dye + H_2O_2 (13)

Determination of triglycerides

Triglycerides are measured using the triglyceride enzymatic method (Glycerol Phosphate Oxidase), which uses the glyceride reagent kit REF 7D74 described by **[20]**. The triglycerides, after several coupled reactions, give a colored complex whose intensity has been spectrophotometer at 500 nm, is proportional to the amount of triglycerides present in the serum. The principle is based on the enzymatic determination of free glycerol after the action of lipase. Triglycerides are hydrolyzed by lipase to fatty acids and glycerol. Glycerol is phosphorylated by adenosine triphosphate (ATP) and glycerol kinase (GK) to give glycerol-3-phosphate and adenosine diphosphate (ADP). Glycerol-3-phosphate is oxidized to Dihydroxyacetone phosphate (DAP) by glycerol phosphate oxidase (GPO) with production of hydrogen peroxide (H_2O_2). In a peroxidase-catalyzed color reaction, H_2O_2 reacted with 4-aminoantipyrine (4-AAP) and 4-chlorophenol (4-CP) to produce Quinoneimine, a red chromophore (which absorbs at 500 nm) with the intensity of the staining measured at 500 nm is directly proportional to the amount of triglycerides in the serum. The principle is summarized in following reactions:

Equation 14 :

$$\text{Triglycerides} + H_2O_2 \xrightarrow[\text{Glycerol kinase}]{\text{Lipase}} \text{Glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[\text{G-3P oxidase}]{} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate (G-3P)} \xrightarrow{} \text{Hydroxyacetone phosphate} + H_2O_2$$

$$4\text{-chlorophenol} + 2H_2O_2 + 4\text{-aminoantipyrine} \xrightarrow[\text{Peroxydase}]{} \text{Quinone imine} + 4H_2O_2 \quad (14)$$

Thus in a tube containing the reaction medium was added 0.1 ml of the sample of the serum to be assayed. After stirring, the tube was incubated at room temperature (16-25 ° C) for 15 min and the absorbance read at 500 nm against a blank.
The concentration of triglycerides in the sample is expressed as indicated in
Equation 15:

$$\text{Concentration of triglycerides (g / l)} = \text{DO Echantillon /DO Standard} \times \text{C.E} \quad (15)$$

C.E = glycerol standard concentration = 2.28 mmol / l, ie 2.28 mmol / l of triglycerides or 2 g / l of toluene.

Hematologicalanalysis

Hematological analysis was performed using an automatic hematological analyzer (Coulter STKS, Beckman). Included parameters: RBC count, white blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), corpuscular hemoglobin mean (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count were determined as described by **[23,24]** .The assay was done from serum on blood samples.

Determinationoftheionogram

The various elements of the ionogram (chlorine) were determined by the colorimetric method, with the exception of sodium and potassium, which were measured with the Hospitex Screen flame spectrophotometer. The dosage was made from the serum on the blood samples.

Statisticalanalysisoftheresults

Using the graph pad software version 5.0.1 (USA), the results have been statistically processed. The test results are expressed on average (m ± esm). The results of biochemical and hematological tests were analyzed by the one-way ANOVA, the Dunnett / Neuman-keuls test for multiple comparisons and determination of significance levels. Values of $P \leq 0.05$ are considered statistically significant.

RESULTS

Phytochemical screening The phytochemical screening of *T.macroptera* stem bark extract, using different standard tests shown in table I.revealed that the aqueous extract showed the presence of polyphenolic compounds, flavonoids, saponins, galic tannins, cardiotonic glucosids. but alkaloids, sterols, terpens , coumarins and cathechic tannins were absents. Also table 3, showed that ethanolic extract contains all the compound of aqueous extract with the presence of sterol and terpens**.**

Table 3: Phytochemical constituents of aqueous and ethanolic70% extracts of stem bark *T. macroptera*

Chemical class	Alc	Polyph	Flav	ST	TGal	TCat	Coum	Sap	Glc
Aqueous extract	-	+	+	-	+	-	-	+	+
Ethanolic70%e extract	-	+	+	+	+	-	-	+	+

Key: Alc: Alcaloides, Polyph: Polyphenols, Flav: Flavonoides, ST: Sterols &Terpens, TGal: Tanin Galique, TCat: Tanin Catechique, Coum: Coumarine, Sap : Saponosides, GlC : Glucosides cardiotoniques

Sub-acutetoxicity of ethanol extract 70%

1. Acute toxicity of ethanol extract 70%

The study of the acute toxicity of 70% ethanolic extract was carried out with rats. Tolerated maximum doses (DMT) 0; 2000 and 5000 mg / kg / mc were used. The results are shown in **Table4.**

No dead rats or signs of intoxication were observed after 24 hours, after administration of 5000 mg / kg / mc which corresponds to DMT. However, some behavioral changes were

observed with the different doses used. All animals survived from 14 days of dosing (DMT) observations between 2000 and 5000 mg / kg / mc, which implies that the LD_{50} is greater than 5000mg/kg/mc.

The study of the sub-acute toxicity of the 70% ethanolic extract made it possible to obtain the results mentioned in Tables **2**,**3, 4, 5, 6, 7 and 8**.

Effect of ethanol extract70% on the behavior of rats after 28 days of gavage

Table 5: shows the behavior of flushes after 28 days of feeding. Analysis of the table reveals that there was a change in the behavior of rats at different doses of extract administered. Thus, at doses of 100 and 300 mg / kg bm, all rats survive and show up, with hair straightening. But with the 300 mg / kg bm dose, the rats lose their appetite with a slowdown in activity.

At the dose of 500 mg / kg bm, in addition to the change in behaviors mentioned above, the rats become weaker.

Table 4: Behavior of rats after 14 days of feeding doses

Doses (mg/kg/mc	Rats (sex)	Number used	Death number	Days	Comments
controls	F	6	0	14	Normal behavior of rats after gavage
2000	F	6	0	14	Hair straightening ⎪ Drowsiness ⎪ No death
5000	F	6	0	14	⎪ Hair Straightening ⎪ Accelerated heart rate ⎪ Drowsiness ⎪ Slow activity ⎪ No death

F = female Number: test number: 3.

Table 5: Behaviorof rats after 28 days of feeding doses

Doses (mg/kg/mc	Rats (sex)	Number used	Death number	Days	Comments
controls	F/M	6	0	28	Normal behavior of rats after gavage
100	F/M	6	0		⎪ Hair Straightening ⎪ Drowsiness ⎪ No death
300	F/M	6	0	28	⎪ Hair Straightening ⎪ Drowsiness ⎪ Slow activity ⎪ Loss of appetite ⎪ No death

500	F/M	6	0	28	⎫ Hair Straightening ⎪ Drowsiness ⎪ Slow activity ⎪ Weakness ⎪ Loss of appetite ⎭ No death

F = female M = mal

Effect of ethanolic extract on body mass of rats for 28 days force-feeding.

The effect of the ethanol extract on rat body mass was assessed from the calculation of the mass change and the results are reported in Table 6. Analysis of the table reveals that there is a mass variation at each dose level. Thus, we obtain:

For the 0 mg / kg bm (controls) dose, the variation is 1 g or 0.5%. For the 100 mg / kg bm (extract) dose, the variation is 23.37 g or 20.02%. -For the 300 mg / kg bm (extract) dose, the variation is 26.99 g or 20.25%. -Finally for the 500 mg / kg bm (extract) dose, the variation is 37.70 g or 23.90%. Moreover, it is noted that this variation corresponds to a decrease in the body weight of the rats and which is dependent on the dose of extract administered. It also appears that the greater the dose, the greater the decrease. Thus the largest decrease is equal to 37.70 g or 23.90% corresponding to the batch of rats that received 500 mg / kg body mass.

Table 6:Body mass variation of rats during 28 days of force-feeding

Extract Doses (mg / kg/ bw)	1st day	7th Day	14th Day	21st Day	28th Day	Mass Variation (g)	Percentage (%)
Witness	177.00 ±0.60	176.00 ±1.58	176.70 ± 1.00	176.70 ± 0.58	176.00 ±1.58	**1**	**0.5**
100	116.70 ±1.73	116.30 ± 1.73	109.00 ±2.73	107.00 ±2.05	93.33 ±1.66	**23.37**	**20.02**
300	133.30 ±1.06	129.30 ±1.73	126.70 ±2.00	115.70 ±1.73	106.31 ±2.73	**26.99**	**20.25**
500	157.70 ±1.00	140.00 ±1.70	141.00 ±1.00	134.70 ±2.00	120.00 ±1.73	**37.70**	**23.90**

Witness = (distilled water)

Effect of ethanolic extract on biochemical parameters, hematological blood levels and the ion profile of rats after 28 days of feeding

Biochemical parameters: The assay of blood biochemical parameters after 28 days of gavage yielded the results Recorded**Table7:**

The analysis of the table reveals that the values of the biochemical markers (Urea, Creatinine, Asat, Alat, LDH, CK) did not vary significantly (at p <0.05) at the different doses used. However, only triglycerides were significantly modified: 0.42 ± 0.01 and 0.35 ± 0.01, respectively, at doses of 100 mg / kg bm and 300 mg / kg bm. These values indicate an increase in the level of blood triglycerides compared to the control rate which is 0.24 ± 0.3.

Hematological parameters

The determination of blood hematological parameters after 28 days of force-feeding yieldedthe results recorded in Table 8
The analysis of the table shows a significant change (at p <0.05) in the number of certain hematological parameters (GB, HC, MCHC, NEU and LYM) at different doses. Thus, for white blood cells (GB), 14.70 ± 1.18 and 15.77 ± 0.35 were observed respectively at doses of 300 mg / kg bm and 500 mg / kg bm. This change results in a decrease in the number of white blood cells compared to the number of tears (20.03 ± 0.58). For the hematocrit it is at the dose of 500mg / kg mc that the modification takes place (38, 90 ± 1, 07), against 34, 33 ± 1, 83 for the control. This change indicates an increase in the number of blood hematocrit. As for the mean corpuscular concentration of red blood cells (MCHC), the change occurs at the dose of 500 mg / kg mc with 35.47 ± 0.52 against 38.73 ± 0.09. This change indicates a decrease in the number of the MCHC. For neutrophils, there was 34.33 ± 2.26 at a dose of 300 mg / kg bm versus 28.67 ± 2.20 for the control. This value indicates an increase in the number of neutrophils, but at a dose of 500 mg / kg bm, the number increases to 28.00 ± 1.36. This reflects the return to the normal reference value (28.67 ± 2.20). Finally, for the lymphocytes, 69.90 ± 1.53 are counted at a dose of 300 mg / kg bm versus 75.67 ± 1.76 for the control. There is a decrease in the number of lymphocytes.

The ionic profile

The ion profile of rats after 28 days of gavage was determined from the content of Na +, K +, and Cl- ions. The results obtained are recorded in **Table 9**
The analysis of the results shows that only the potassium (K +) ion content was significantly (at p <0.05) modified at different doses. Thus, at the dose of 300 mg / kg bm and 500 mg / kg bm, the respective contents were obtained 3.86 ± 0.86 and 4.00 ± 0.05 against 3.43 ± 0.14 for the control. . This modification indicates an increase in the content of potassium ions in the blood serum. The other two parameters (Na + and Cl-) did not undergo any significant modification.

Table 7: Analysis of biochemical parameters after 28 days of force-feeding

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Parameters	0 mg / kg /bm	100 mg / kg /bm	300 mg / kg bm	500 mg / kg bm

UREA(g / l)	0.16 ± 0.00	0.18 ± 0.01 ns	0.15 ± 0.00 ns	0.17 ± 0.00 ns
CREAT(mg / l)	7.33 ± 0.33	7.33 ± 0.33 ns	6.66 ± 0.33 ns	8.00 ± 0.57 ns
ASAT orTGO (UI)	14.67 ± 0.66	13.42 ± 0.57 ns	13.53 ± 0.33 ns	13.65 ± 2.72 ns
ALAT or TGP (UI)	11.67 ± 1.20	11.00 ± 0.57 ns	11.16 ± 0.33 ns	11.32 ± 0.57 ns
GLC (mg / dl)	0.5 ± 0.01	0.60 ± 0.01 ns	0.53 ± 0.57 ns	0.40 ± 0.04 ns
CHL (g / l)	0.61 ± 0.12	0.60 ± 0.03 ns	0.62 ± 0.01 ns	0.62 ± 0.21 ns
TRIGL (g / l)	0.24 ± 0.03	**0.42 ± 0.01 ***	**0.35 ± 0.01 ***	0.23 ± 0.00 ns
LDH (g / l)	l 103.32 ± 1.58	101.7 ± 1.85 ns	102.70 ± 4.25 ns	102.00 ± 3.51 ns
CK (g /	15.67 ± 1.20	13.33 ± 1.45 ns	14.33 ± 1.45 ns	14.47 ± 0.88 ns

*** (significant, p <0.05); ns (not significant). Data are expressed as mean plus or minus standard error on average: M ± wk. n = 3 (number of trials)**

Table 8: Analysis of hematological parameters Settingshematologic

Parameters	**0 mg / kg /bm**	**100 mg / kg /bm**	**300 mg / kg bm**	**500 mg / kg bm**
GB	20.03 ± 0.58	18.30 ± 1.04 ns	**14.70 ± 1.18 ***	**15.77 ± 0.35 ***
GR	6.72 ± 0.23 6.43	6.43 ± 0.89 ns	6.89 ± 0.11 ns	6.97 ± 0.41ns
HB	13.00 ± 0.79	13.70 ± 0.36 ns	13.50 ± 0.50 ns	14.50 ± 0.60 ns
HC	34.33 ± 1.83	33.97 ± 1.90 ns	33.17 ± 0.10 ns	**38.90 ± 1.07 ***
VGM	51.17 ± 0.87	52.83 ± 1.51 ns	52.33 ± 1.16 ns	52.20 ± 2.60 ns
CMH	19.60 ± 0.55	19.43 ± 0.74 ns	19.63 ± 0.35 ns	19.17 ± 0.36 ns
CCMH	38.73 ± 0.09	39.10 ± 1.45 ns	38.27 ± 0.22 ns	**35.47 ± 0.52 ***
PLT	579.30 ± 17.07	569.30 ± 12.39ns	582.30 ± 10.1 ns	592.70 ± 18.3ns
NEU	28.67 ± 2.20	30.00 ± 2.36 ns	**34.33 ± 2.26 ***	28.00 ± 1.36 ns
LYM	75.67 ± 1.76	75.00 ± 3.79 ns	**69.90 ± 1.53 ***	75.47 ± 4.04 ns

*** (significant, p <0.05); ns (not significant). The data are expressed by mean plus or minus standard error on the mean: M ± esm. N = 3 (test number).**

Table 9: Ionic profile of rats after 28 days of feeding.

parameters	**0 mg / kg bm**	**100 mg / kg /bm**	**300 mg / kg bm**	**500 mg / kg bm**
Sodium (Na +) meq / l	137.00 ± 0.57	136.70 ± 0.33 ns	137.00 ± 1.15 ns	137.00 ± 0.88 ns
Potassium (K +) meq / l	l 3.43 ± 0.14	3.33 ± 0.08 ns	**4.86 ± 0.08 ***	**5.00 ± 0.05 ***
Chlorine (Cl-) meq / l	97.67 ± 0.88	97.00 ± 0.57 ns	99.00 ± 0.57 ns	98.33 ± 1.85 ns

*** (significant, p <0.05); ns (not significant). Data are expressed as mean plus or minus standard error on average: M ± wk. N = 3 (test number).**

Discussion

The good antibacterial activity and the phytomolecule richness of the ethanolic extract led to a verification of the safety of this extract through a study on rats. Thus, at doses of 2000 and 5000 mg / kg / bm, the slow-down in activity was observed: the acceleration of the heart

rate and a weakness in rats. These changes in behavior of the rats are probably due to the involvement of the central nervous system.

But our phytochemical study does not confirm the presence of alkaloids in our extracts which are known for their neurological effects, in particular the quinolizidine alkaloids, whose effects are associated with several activities such as hypoglycemia, hypertension and respiratory depression**[25].** Also the weakness in the rats, after 14 days of force-feeding, could be explained either by the refusal to eat, or by a disturbance, at the level of the physiological equilibrium.According to Fourrier **[26]**, the invasion of cellular tissue by lipid compounds (fats) causes inhibition elimination of hepatic triglycerides in the blood. Administration to rats of a single dose of 70% ethanolic extract of T. macroptera orally revealed a lethal dose (LD_{50}) greater than 5000 mg / kg / bm. But in our case study, up to the maximum dose of 5000 mg / kg bm, all animals survive. Which leads us to say that the LD_{50} is greater than 5000 mg / kg of bm.

Thus Based on the OECD classification standard **[27]**, 70% ethanol extract would belong to category 5 (LD_{50} greater than 5000mg / kg bm) and therefore considered an oral non-toxic substance. Other authors have used the same method (limit test proposed by OECD), and also found an LD_{50} greater than 5000 mg / kg bm, this is the case ofAdeneyé and his scientific collaborator**[28]**. There are obtained an LD_{50} greater than 5000 mg / kg bm after administration of the aqueous extract of Cymbopagon citratus (Poaceae) to rats after 14 days of gavage.
Therefore ethanol extract did not result in the death of the rats following a high dose of 5000 mg / kg bm.

To assess the effect of bioaccumulation of the active ingredients of the extract, the rats are subjected to another test which is the test of sub-acute toxicity during 28 days.Administration to rats at doses of 100, 300 and 500 mg / kg bm during 28 days did not record any dead rats in the sub-acute toxicity test rats. However, at the level of general behavior, the rats receiving doses of extract are less active and doze so much compared to the control rats.

The change in body mass during the experiment showed that there was a decrease in body mass throughout the 4 weeks compared to controls. The body mass variation is used as an indicator of the adverse effects of the chemical compounds contained in our extract **[29]**, because the loss of mass is correlated with the physiological state of the animal. This drop in mass can be explained by a reduction in food consumption but also by the possibilities of dose / absorption interactions and the decrease in the amount of food intake. Other studies have also shown a decrease in the oral mass of rats of an extract of Chicococea abla**[30]**. Finally, it appears that the decrease in body mass during the 28 days of daily treatment suggests that the subchronic and oral administration of the ethanol extract 70% of T. macroptera would have effects on the growth of whistar rats.

This study was also the subject of an analysis of the haematological and biochemical parameters of the experimental rats after 28 days.
The hematopoietic system is one of the most sensitive targets for toxic compounds and an

important indicator of the physiological and pathological condition of humans and animals. So any change in the hematopoietic system is of greater valuepredictive for human toxicity when data are derived from animal studies **[31]**. In this respect, the state of bone marrow activity and intravascular effects were monitored by haematological examination. Our results showed a decrease in the white blood cell count and the average corpuscular concentration of red blood cells, then an increase in the rate of hematocrits. This decrease in the leucocyte rate could be explained by a decrease in the production of regulatory elementsof Hemopoiesis such as CSF (colony stimulation factor) and TPO (trombopoietin) by macrophages and stromal cells of the bone marrow, thus providing an unfavorable environment for hematopoiesis**[32]**. In addition, this decrease in white blood cell counts is followed by a decrease in the mean corpuscular concentration rate in rats, reflecting a decrease in hemoglobin concentration and thus favoring the emergence of hypochromic**[33]**. In addition, the increase in the hematocrits rate is due to the presence of certain chemical constituents of our extract, especially fatty acids.The presence of fatty acids in the blood, inhibit the hemolysis of erythrocytes induced by singlet oxygen **[34]**.

Regarding the biochemical results, the ethanolic extract had no effect on the serum levels ofurea and creatinine. This suggests that the ethanolic extract of *T macroptera* does not alter the structure and function of the kidneys, which retains their structural integrity. Serum urea remains a gross screening parameter for renal diseases and in nephrological pathology. It is always associated with dosage Creatinine. These parameters are significantly elevated in the event of impaired renal structure and function **[35].**

Moreover, the serum level of the transaminases TGO or ASAT and TGP or ALAT did not undergo any significant variation and this good availability of transaminases in the blood of the experimental rats would be due to the presence of betulin resulting from the ethanolic extract administered.Thus, According to Tang**[36]**, betulin, in addition to its inhibitory action on germs, normalizes the level of Alanine Aminotransferase (ALT) in the blood. Which means in other words that the liver and muscles to a lesser degree have not been reached.
The liver is the first target of toxicity and the first organ exposed to all that is absorbed in the small intestine; it metabolizes foreign substances or compounds that may be hepatotoxic **[37]**. The liver works in combination with the kidneys to remove toxic substances from the blood**[38]**. Thus, any necrosis of liver cells, leads to a significant increase of ASAT and ALAT enzymes in the blood serum **[39]**. In addition, the elevation of transaminases in the blood is an index of damage to the parenchymal cells of the liver **[40]**.
However our results indicate a significantly elevated triglyceride levels in the rats administered to the ethanol extract compared to the control. Indeed, the ethanolic extract has a two-phase effect on blood triglycerides. An increase in the rate at 100 and 300 mg / kg bm followed by a decrease at 500 mg / kg / bm which tends to normalize with the control. This phenomenon could be attributed to a problem of resorption of the experimental extract. Also, the irregularity of triglyceride levels, could be explained by an irregularity of the presence of certain active principles of our extract in the blood of experimental rats, hence the possibility of disruptionof the transfer process. Thus, according to Sakly, the tannins across the membrane of animal cells and by their astreingent property, reduce the permeability of the mucous membranes and vessels**[41]**.

In addition, the elevation of triglyceride levels would be due to the inhibition of the elimination of triglycerides hepatica in the blood that subsequently invade the cellular tissues **[26].**

With the exception of potassium, the ethanolic extract did not significantly alter the ion profile of the administered rats. The potassium level was elevated compared to the control. This increase in serum potassium is due to the richness or the abundant presence of potassium in our ethanolic extract. The work ofscientifics authorsconfirms this thesis**[42]**. In fact, these authors determine, during the determination of minerals in the powder of *T.macroptera* bark, the contents of 6864 ppm for potassium against 149.5 for sodium and 0 ppm for chlorine. In addition, the elevation of K^+ ion level in the blood could be explained by a failure or blockage of the Na^+ / K^+ ATPase pump, thus causing a massive diffusion of K^+ ions in the extracellular medium. Finally, the results obtained from the acute and sub-acute toxicity of the ethanolic extract70% of *T. macroptera* show that this substance has no toxic effect on the ion profile, on the biochemical and haematological parameters of the rats tested at the indicated doses.

Conclusion

The acute and sub-acute toxicity study of *T. macroptera* showed no serious symptoms of toxicity. The LD_{50}> 5000 mg / kg body weight indicates that the ethanolic extract of *T. macroptera* bark is non-toxic. However, the administration of the ethanolic extract on animals tested orally for 28 days not only causes a change in body mass, but also a disturbance in some biochemical parameters, hematological and ionic profile. In addition, the study of sub-acute toxicity notes that, with the results obtained, there was no change in transaminases, urea creatinine, CK and LDH. This suggests that the liver, heart and kidneys have not been altered.

Conflicts of Interest

The authors declare no conflicts of interest regarding this manuscript. The authors alone are responsible for the content and writing of the manuscript

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