Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2018; 10(3); 92-97

ISSN: 0975-4873

Research Article

Phytochemical and Toxicological Studies of An Aqueous Trunk Bark Extract of *Parkia biglobosa* (Jacq.) Benth (Mimosaceae)

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Received: 20th Nov 17; Revised 9th Jan, 18, Accepted: 15th Feb, 18; Available Online:25th Mar, 18

ABSTRACT
Among the plants commonly used in the traditional African pharmacopoeia, *Parkia biglobosa* called 'nere' in the West African sub-region is one of the most common. We decided to determine the essential components of the aqueous extract of the bark trunk of the plant and to carry out an acute toxicity study. The phytochemical study of the trunk bark of this plant revealed the presence of sterols, polyterpenes, polyphenols, catechic tannins, alkaloids, flavonoids and saponosides. At the concentration of 2 mg/ml or 40 mg/kg body weight (bw), all animals fully regained their motive power and balance after one hour and throughout the experiment, no animal die (0%) by intraperitoneal injection. At 10 mg/ml, equivalent to 192 mg/kg bw. AEPB became toxic. The doses of 192, 380 and 400 mg/kg bw gived respectively 20, 40 and 60% mortality. At 40 mg / ml equivalent to 851 mg/kg bw, all mice died, the LD100 was reached, with the 100% of death. AEPB is a moderately toxic plant whose use in pharmacopoeia must be controlled.

Keywords: *Parkia biglobosa*, phytochemical study, toxicity.

INTRODUCTION

Parkia biglobosa (Jacq.) Benth., is a plant of the family of Mimosaceae. In Côte d'Ivoire, it is called "nere" in Malinke, "kparale" or "kpale" in Baoule. It is a tree of 10 to 13 m of height, with short barrel, cylindrical, robust. The stem bark, dark, is deeply striated. The use of 'nere' for the treatment of various diseases has been reported by several authors. It is therefore a species known for its numerous virtues in the African Pharmacopoeia. Its various parts are used, alone or in combination, in various medicinal preparations. The fresh leaves, crushed and macerated in water, are used in the treatment of hemorrhoids, ascariasis, colds, pertussis and yaws, amoebic dysentery, conjunctival haemorrhage, palpitations, treatment of shingles, Edema, bronchitis, herpes, hemorrhoids and leucorrhoea[1,2,3]. Consumed on an empty stomach, it is a vermifuge (tapeworm) to treat cataract[4]. This part of *Parkia biglibosa* is used to combat jaundice[5].

The barks are used as a drink or for ablutions during a febrile access, or to clean the wounds[6]. It is used for the treatment of leprosy, jaundice, and pneumonia[7]. They are also used to treat measles, chicken pox, peptic ulcers, diarrhea and cardiac disorders[8]. The fruit is used to prepare the "soumara" that accompanies the Senegalese rice called "tchep-djene" and is considered an antihypertensive agent[9,10,11]. It is used of the case of abortion threats, osteopathies, odontalgias, mumps, hemorrhoids, dermatoses[12]. It intervenes in the treatment dental caries, rectal prolapse and vaginal pruritus[13,14].

It is also used in treatment of inguinal hernia, jaundice, polyuria, amenorrhea, fibroma, enuresis, pertussis, epilepsy and bilharziasis[15,16]. In view of this, the aim of this study is to carry out a phytochemical study of the aqueous trunk bark extract of Parkia biglobosa and to undertake a test of acute toxicity of this extract in mice.

MATERIALS AND METHODS

Biological material

Animal material

The toxicological tests (acute toxicity) are carried out with white mice, male and female of specie Mus musculus, strain Swiss. They come from the Animal Physiology Laboratory of the Training and Research Unit (UFR) of Biosciences of the Felix Houphouet-Boigny University (Former University of Cocody). They had access to food and water *ad libitum*. They benefited from the light of day and the darkness of the night (12 hours/12 hours). They weighed an average of 22 ± 3.1 g. All procedures are in accordance with the guide for the health and use of laboratory animals published by the National Institute of Public Health.

Vegetal material

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Table 1: Phytochemical component of the trunk bark of *Parkia biglobosa* (Jacq.) Benth.

Chemical groups		Reagents	Ether solution	Methanol solution	Aqueous solution
Quinones		Borntraegen	-	-	-
Tannins	Catechics	Stiasny	-	-	+
	Gallic	Sodium acetate and $FeCl_3$	-	-	-
Alkaloids		Dragendorff et Bouchardat	+	+	+
Sterols et polyterpenes		Liebermann	+ +	+	+
Polyphenols		Ferric Chloride	-	+	+
Flavonoids		Cyanidine	-	+	+
Saponosides		Physical	-	-	+ +

Appreciable amount (+ + +); Average quantity (+ +); Traces (+); Complete absence (-).

Table 2: number and percentage of mice dead as a function of AEPB injected dose

Mice lots numbers	Concentrations of AEPB injected (mg/ml)	Doses equivalents mg/kg b.w.	Number of mice dead/lot	Percentage of mortality
1	2	40	0	0
2	10	192	2	20
3	20	380	4	40
4	25	400	6	60
5	32	645	9	90
6	40	851	10	100

The bark of *Parkia biglobosa* (Jacq.) Benth. (Mimosaceae) was collected behind the Amphitheater C of the UFR Biosciences of the Felix Houphouet-Boigny University (Former University of Cocody), Abidjan, Côte d'Ivoire. Authentication was made by Professor AKE ASSI Laurent, thanks to the herbarium samples 10933 of 22 -12-1969, 13329 of 8-02-1976 and 13336 of 9-02-1976 of the National Center of Floristic (CNF), of Côte d'Ivoire. The bark is cut into small pieces, dried in the sun and then crushed in a mechanical ball mill for at least an hour. A sufficiently fine powder of brown color is obtained. Fifty grams (50 g) of ground material are mixed in 1 liter of distilled water under slow magnetic stirring for 24 hours. The solution obtained is filtered on hydrophilic cotton on Wattman filter paper according to the method described by Kouakou et al.[17]. The filtrate collected in a flask is then evaporated under vacuum at 90 °C, using a rotary evaporator of the rotavapor type and dried in an oven at 70 °C (Kouakou et al.[17]). A perfectly water-soluble fine brown powder, the crude aqueous extract of the bark of *Parkia biglobosa* (AEPB), was obtained and kept in the fridge. A stock solution from which the experimental solutions will be made with the Mac Ewen (ME) is prepared.

Phytochemical screening

The detection of secondary metabolites in the aqueous trunk bark extract of *Parkia biglobosa*, was carried out according to the technique of interpretation of the reactions. To this end, various known reagents are added to the aqueous solution of *Parkia biglobosa* and the quality of the reaction obtained makes it possible to conclude as to the presence of the desired compound or not. We use the characterization method known and approved by Belemtougri et al.[18].

Acute Toxicity Method

Groups of 7 batches of ten (10) mice were obtained.

Lot No. 1 serves as a control. The mice of this batch receive by injection, 1ml of physiological solution of Mac Ewen type.

The mice of batches Nos. 2 to 7 receive respectively 2, 10, 20, 25, 32 and 40 mg/ml equivalent to 40, 192, 380, 400, 645 and 851 mg/kg bw of AEPB by intraperitoneal.

Determination of the LD50 by the Graphical Method of Miller and Tainter[19]

At the end of the twenty-four (24) hours after the injection of the different doses of AEPB, the dead number is counted and the mortality percentage per lot is calculated. The curve of the percentages of dead mice per batch (mortality) as a function of the logarithm of the injected EAPB dose is then established.

The calculation method of Dragsted and Lang[20]

This method is based on the following assumption

- any animal that has survived a given dose of a substance administered to it could have survived any other lower dose of that substance (or of that substance);
- Similarly, any animal which has succumbed to a given dose of a substance administered to it would have succumbed to any other higher dose.

Thus, the mortality rate (M %) for a given dose of the administered substance is given by the number of dead specimens (Nm) at that dose, the number of dead specimens plus the number of survivors (Nv).

M% = Nm X 100 / Nm + Nv

The calculation of the LD_{50} according to the Dragsted and Lang method is done by extrapolation as the search for the approximate dose value corresponding to 50% mortality in an interval (X1-X2).

The formula is as follows:

LD_{50}= 50 (X2-X1) + (X1Y2-Y1X2) / (Y1-Y2)

- X1: lower dose framing the LD_{50};
- X2: upper dose framing the LD_{50};

Y1: percentage mortality corresponding to X1 (M %);

- Y2: percentage of mortality corresponding to X2(M %).

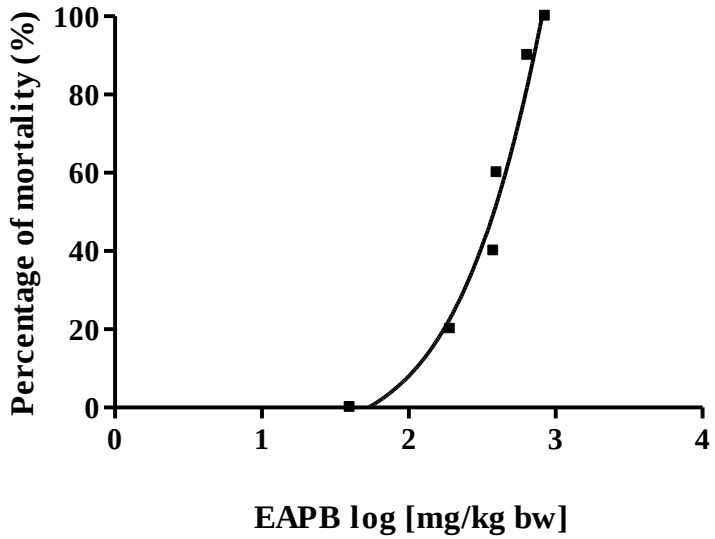

Figure 1: Percentage of mortality of mice as a function of the logarithm of EAPB dose.

$$y = 74.99 * \log x - 133.4$$

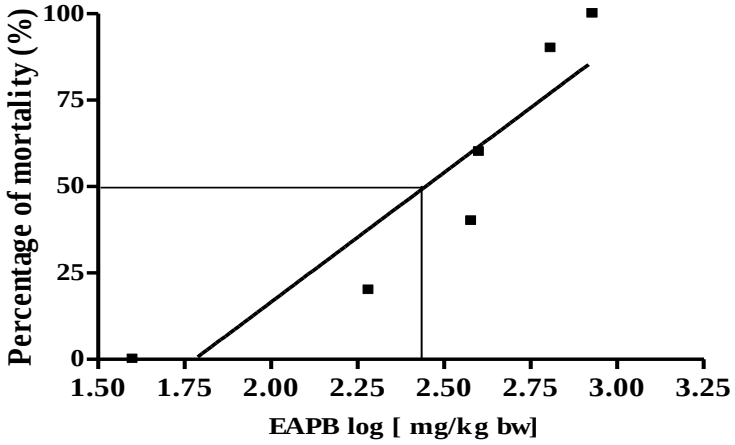

Figure 2: Linearized curve of percentage of mortality in log (mg/kg bw).

Statistical analysis

The results are analyzed using the GraphPad Instat software ANOVA variance and the Tukey-Kramer multiple comparison test where $p < 0.05$ is considered significant. The values are obtained with the standard error of mean. GraphPad prism 5 software, was used to draw the curves.

RESULTS

Phytochemical screening

The phytochemical study of the trunk bark of *Parkia biglobosa* carried out in the laboratory of phytochemistry and medical materials of the department of pharmacognosy of the UFR of the Pharmaceutical and Biological Sciences of the Felix Houphouet-Boigny University (Former University of Cocody), Abidjan, revealed the presence of sterols, Polyterpenes, polyphenols, flavonoids, catechic tannins, alkaloids and saponosides (Table 1).

Acute toxicity study of AEPB in mice

Behaviors of the mouse under the effect of AEPB

AEPB, at increasing concentrations ranging from 2 mg/ml to 40 mg/ml, changes the behavior of mice after three (3) minutes (for high doses) and ten (10) minutes (for low doses).

Indeed, in each batch, all the mice exhibit a progressive decrease of the motor activity, characterized by a difficult displacement. The animals drag their rear train, which is then particularly low. At times they snuggle into a corner of the cage.

From 32 mg/ml, in addition to the behaviors above observed, torsions of the body of the mouse are noted. Thereafter, the motor activity of the animal returns to normal but earlier for mice subjected to low doses of AEPB and later to those that do not succumb to the high doses.

During the observation period, all animals recover their motor capacity and equilibrium at the end of one (1) hour

for the concentration of 2 mg/mg or 40 mg/kg bw. The recorded mortality was observed approximately fourteen (14) hours after the injection for the highest dose (851 mg/kg bw) for which all mice died. For lower doses, the duration of death is longer.

Determination of the AEPB LD50 by the graphical method

At the end of the twenty-four (24) hours after the injection of the different doses of AEPB to mouse, the number of dead mice is raised and the percentage of death per batch, that is to say mortality, is calculated (Table 2). The data in the table are averages of three tests.

The curve of the percentages of dead mice per batch (mortality) as a function of the logarithm of the dose of injected AEPB presents a sigmoid appearance with a maximum effect (Figure 1).

A part of this curve can be assimilated to a straight line, that is to say the slope. This line, which expresses the mortality of the mice as a function of the logarithm of the dose of AEPB, enabled the LD50 to be determined graphically. The value of this dose is given by the following equation: y = 74.99logx - 133.4. The calculated dose is then 282 mg/kg of body weight.

Determination of the LD50 by the calculation method of Dragsted and Lang

The doses that surround 50% mortality are between 380 mg/kg bw. and 400 mg/kg bw. The formula of Dragsted and Lang makes it possible to obtain by calculation the value of the LD 50 which is 390 mg/kg bw

DISCUSSION

Phytochemical analysis of the aqueous trunk bark extract of *Parkia biglobosa* (EAPB) showed that it contains qualitatively, sterols, polyterpenes, polyphenols, flavonoids, catechic tannins, alkaloids. These substances could be at the origin of the pharmacological effects of this plant used in traditional medicine to treat many diseases. Indeed, all chemical compounds of the aqueous trunk bark extract of *Parkia biglobosa* (EAPB), are endowed with antimicrobial activity[21]; which justifies its use in traditional medicine for the treatment of many infections. In addition, sterols, polyterpenes and polyphenols have antipyretic and analgesic properties[22]. According to Mc Namara[23], sterols and polyterpenes have necrotic and cytotoxic properties in rodents. As for saponosides, they have a haemolytic action explaining the toxic effect of some of them[24] whereas alkaloids cause bradycardia[25,26]. Several authors[27,28,29,26,30,31,32], showed the beneficial effects of phenols and flavonoids on the cardiovascular system of laboratory animals through their cardio-inhibitory, vasodilatory and hypotensive activities. It is likely that the presence of alkaloids, polyphenols and flavonoids in EAPB is a serious indicator for pharmacological activities on the cardiovascular system.

The data transcribed on the table following acute toxicity tests show that the pharmacological effect of AEPB is dose-dependent. Changes in the status of mouse activity up to survival or death in relation to AEPB doses are factors that establish the compliance and reliability of the acute toxicity study design. Indeed, this method is similar to that used by many authors and taken up by WHO[33]. The dose-related mortality curve is sigmoid and shows on the one hand, that the effect of AEPB is dose-dependent and, on the other hand, that its activity passes through receptors.

Moreover, this effect is proportional to the number of receptors or sites occupied. The slope of the graph was used to determine an LD_{50} between 282 mg/kg bw.

The Dragsted and Lang calculation method, gave an LD_{50} of 390 mg/kg of bw.

The exact value of the LD 50 in our experimental conditions is in the range of 282 to 390 mg/kg bw. This range is the similar to that established by Millogo et al.[34] on bark of *Parkia biglobosa* which is between 250 to 500 mg/kg bw.

According to the classification of Diezi[35], the pharmacological substances with an LD_{50} between 5 mg/kg bw. and 5000 mg/kg bw. are classified as moderately toxic. Since the AEPB LD_{50} is between 282 to 390 mg/kg bw, this substance can be considered a moderately toxic substance. The presence of saponosides in EAPB can justified its moderate toxicity when it is administered intraperitoneally in mice.

CONCLUSION

The phytochemicals studies showed the presence of flavonoids, catechic tannins, alkaloids, saponosides, sterols, polyterpenes and polyphenols in AEPB. These different chemical groups are substances commonly used in therapeutics which, because of their properties, are responsible for the pharmacological effects of this plant used in traditional medicine to treat many diseases. Toxicologicals study revealed that AEPB is a moderately toxic substance. This toxicity of AEPB cannot be a brake on its use for therapeutic purposes because all pharmacodynamic substances are toxic when the doses administered are sufficient. However, this substance must use with precautions.

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