ORIGINAL RESEARCH

IN VIVO TOXICITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF TWELVE ANTIMALARIAL PLANTS

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ABSTRACT:

The practice of traditional medicine by plants is very popular among African populations including those of the Department of Agboville (South-East of Côte d'Ivoire) against various diseases, particularly malaria. The objective of this study is to verify by subacute toxicity the safety of the aqueous and ethanolic extracts of twelve medicinal plants from this Department in order to justify their traditional uses. The methodology used is OECD guideline 423, which is to determine in which dose range a substance should be considered lethal. Thus after the distribution of the female rats in several lots of 3, we gave them different doses (5, 50, 300, 2000 and 5000 mg/kg per body weight (PC)) of our aqueous and ethanolic extracts. The results obtained at the end of the experiment give a lethal dose 50 (LD_{50}) estimated to be greater than 5000 mg/kg PC. This means that the extracts of the 12 plants administered as a single dose by oral see result in a relatively low toxicity even if we have noticed some slight signs of intoxication at the limit dose of 5000 mg/kg PC.

At the end of this study, the estimated LD_{50} greater than 5000 mg/kg PC makes it possible to affirm the safety of twelve plants used by the traditional health practitioners of the Department of Agboville (South-East of Côte d'Ivoire).).

KEYWORDS: Antimalarial, Lethal dose, Traditional medicine, Toxicity

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INTRODUCTION

People has always used plants for multiple purposes that is to say food, pharmacological, cosmetic ... Many scientific studies have allowed the discovery of bioactive compounds of medicinal plants. Unlike synthetic drugs, medicinal plants not only provide an active ingredient but a multitude of compounds with complementary therapeutic effects, forming a balanced biochemical complex[1]. They have a softer action and act in depth to balance the body by stimulating the natural defenses also. In Africa, about 80% of the population uses plants to heal[2].

Although herbal medicine is accepted, effective, less expensive, available and with low toxicity, care must be taken in their use, because often the abusive and uncontrolled use of these plants whose safety is not ignored could expose individuals to other conditions despite the presence of their active ingredients. In fact, the doses used for the various traditional treatments are imprecise, that's why toxicological studies are done on medicinal plants to avoid the real risks of therapeutic accidents that can sometimes be dramatic[3,4].

The purpose of this study is to evaluate the acute toxicity of 12 plants, traditionally used for the treatment of malaria in Côte d'Ivoire, according to the OECD guideline 423 for the testing of chemicals.

MATERIAL AND METHODS

Material:

The plant material used concerns all the organs of selected antimalarial plants (Table 1). The animal material used consisted of nulliparous and non-pregnant female rats of Wistar strain aged from 7 to 13 weeks and weighing between 184 g and 215 g.

Methodology :

Preparation of plant extracts:

The plant organs were dried out of the sun during two weeks at room temperature and then reduced to a fine powder using a mechanical grinder. The extraction was done according to the method of Zirihi*et al*[5]. One hundred grams of powder were dissolved in 1 L of distilled water using a Blender Mixer. The homogenate obtained was first spun in a square of tissue then filtered successively three times on hydrophilic cotton and once on Whatman paper 3 mm. The filtrate obtained was dried in anincubator at 45°C. The dried extracts obtained constituted our aqueous extracts. The same operation was repeated with 70% ethanol as solvent to give the ethanolic extracts. This allowed us to have 24 extracts including 12 aqueous extracts and 12 ethanolic extracts.

Acute Oral Toxicity Test:

The acute oral toxicity test used here is that described in OECD Test Guideline 4234[6]. It is a method by acute toxicity class to determine in which range of doses of a substance should be considered lethal (LD_{50}).

Animal conditioning

The rats were acclimatized to the conditions of the animal house for 72 h before the treatment at the Laboratory. They were placed in aerated metal cages containing litters of wood chips regularly renewed. Tap water was served to them as a drink and their foods consisted of pellets.

Concentrations of weight of extracts administered to animals

Let P (g) be the weight of the animals.

Let Q (g) be the quantity of extract to be administered corresponding to the dose D (mg / kg of body weight) for a weight rat P :$Q = DxP$

Let V (mL) be the volume of the extracts administered to the rats: $V = \frac{1}{100xP}$

The different Cp (g / mL) weight concentrations of the extracts to be administered are made according to the following formula: $Cp = \frac{PmxD}{V}$ where Pm is the average weight of the rats.

Administration of test substance by gavage

The night before the tests, rats were deprived of food but no water. In the morning, the fasted rats were weighed and the test substance administered orally in a single dose by gavage using an esophageal cannula. After feeding the extract, the animals are returned to their cages where they could access food again 3-4 hours later. For the administration of each extract we made a batch of 3 female rats for the initial trial and possibly another batch of 3 rats for the confirmation test when there were 0 or 1 deaths. If this result remained the same, we would switch to the next lower and/or higher dose. Then we went to the limit dose of 2000 mg/kg PC then 5000 m /kg PC when the lower doses had not led to death. Particularly for the case of the limit test for the dose of 5000 mg/kg PC, only a batch of 3 female rats was used to conclude.

Table 1: Organs and method of administration in traditional medicine

Species	Families	Organsused	Method of preparation	How to use
Adenialobata	Passifloraceae	stem Bark	Décoction	Drink
Cola gigantea	Sterculiaceae	stem Bark	Decoction/ kneading	Drink, Bath
Entadamannii	Mimosaceae	stem Bark	Decoction	Drink
Entandrophragmaangolense	Meliaceae	stem Bark	Decoction	Drink
Griffoniasimplicifolia	Caesalpiniaceae	leaves	Decoction/ kneading	Bath
Jatrophacurcas	Euphorbiaceae	stem Bark	Macération	Bath
Landolphia heudelotii	Apocynaceae	leaves	Decoction	Drink, Bath
Mitragynaledermannii	Rubiaceae	stem Bark	Decoction/ kneading	Drink
Parkia bicolor	Mimosaceae	stem Bark	Decoction/ kneading	Drink, Bath
Spathodeacampanulata	Bignoniaceae	stem Bark	Decoction/ kneading	Drink, Bath Purge
Uapacaguineensis	Euphorbiaceae	stem Bark	Decoction/ kneading/infusion	Drink, Bath Purge
Vernonia amygdalina	Asteraceae	stem Bark	Decoction/ kneading	Drink, Bath Purge

Observations

After treatment, the rats were observed individually at least once during the first 30 minutes and regularly during the first 24 hours after treatment, with particular attention during the first 4 hours in order to identify possible deaths and/or the syndrome. Intoxication (tremor, convulsion, salivation, diarrhea, lethargy, sleep and coma). They were then observed daily for 14 days after administration of extracts.

Determination of LD_{50}

The lethal dose 50 or LD_{50} corresponds to the dose that kills 50% of the animals subjected to the toxicological experiment.

For a batch of 3 rats each of which receives a single dose:

- If there is 0 or one of the three animals, the LD_{50} value is estimated to be greater than this dose.
- If 2 or 3 animals die at this dose, we directly define the LD_{50} attached to this same dose.

RESULTS

Observation of the rats immediately after oral gavage (during the first 24 hours) of the extracts at different doses and during the following 2 weeks showed no poisoning or death syndrome (Table 2). Note, however, at the dose of 5000 mg/kg some signs of intoxication (drowsiness, convulsion, accelerated breathing, difficult motor skills and a huddle in a corner of the cage) were noticed during the first 30 minutes of observation. According to the protocol used we concluded that the LD_{50} was estimated to be greater than 5000 mg/kg PC.

Table 2: Result of acute general toxicity in rats following a single dose of extracts

Species	Extracts	Doses given to 3 rats (mg/kg PC) and the number of deaths								
		5	5	50	50	300	300	2000	2000	5000
Adenialobata	Aq	0	0	0	0	0	0	0	0	0
	eth	0	0	0	0	0	0	0	0	0
Cola gigantea	Aq	0	0	0	0	0	0	0	0	0*
	eth	0	0	0	0	0	0	0	0	0
Entadamannii	Aq	0	0	0	0	0	0	0	0	0*
	eth	0	0	0	0	0	0	0	0	0*
Entandrophragmaangolense	Aq	0	0	0	0	0	0	0	0	0*
	eth	0	0	0	0	0	0	0	0	0*
Griffoniasimplicifolia	Aq	0	0	0	0	0	0	0	0	0
	eth	0	0	0	0	0	0	0	0	0
Jatropha curcas	Aq	0	0	0	0	0	0	0	0	0
	eth	0	0	0	0	0	0	0	0	0
Landolphiaheudelotii	Aq	0	0	0	0	0	0	0	0	0
	eth	0	0	0	0	0	0	0	0	0
Mitragynaledermannii	Aq	0	0	0	0	0	0	0	0	0
	eth	0	0	0	0	0	0	0	0	0
Parkia bicolor	Aq	0	0	0	0	0	0	0	0	0
	eth	0	0	0	0	0	0	0	0	0
Spathodeacampanulata	Aq	0	0	0	0	0	0	0	0	0
	eth	0	0	0	0	0	0	0	0	0
Uapacaguineensis	Aq	0	0	0	0	0	0	0	0	0
	eth	0	0	0	0	0	0	0	0	0
Vernoniaamygdalina	Aq	0	0	0	0	0	0	0	0	0
	eth	0	0	0	0	0	0	0	0	0

Eq: Aqueous extract, Eeth: Ethanolic extract,
0: no dead rat and no sign of intoxication at observation
0 *: no dead rat but showing signs of intoxication at observation

DISCUSSION

The study of the acute toxicity of these twelve plants used in the traditional treatment of malaria in the Department of Agboville[7], revealed to us in what range of doses these plants should be considered lethal. The LD_{50} is estimated to be greater than 5000 mg/kg per body weight because no mortality was observed during the trials up to this dose. These extracts given to the rats are "almost non-toxic" according to the toxicity index of the Hodge and Sterner scale[8].

For this LD_{50} value obtained in rats, people of 20 kg or 60 kg should receive respectively 100,000 mg or 300,000 mg of our extracts in a single dose to run the same risk of poisoning as these female rats. This reassures because usually the quantities of extracts prepared by kneading, decocting, maceration or infusion by traditional healers to treat malaria are much lower than these quantities.

Depending on the toxicity class of the Gosselin[9], this dose of 100 g or 300 g of extract is "very little toxic" for humans.

The poverty of these plants in water-soluble tannins[10]may explain their low toxicity[11].

However, according to the Globally Harmonized Classification System (GHS) category, our extracts are in hazard category 5, that is, the acute toxicity is relatively low but may, under certain conditions (age, sex, animal species) , be dangerous for vulnerable populations[5]. This is why Kim *et al*[12]states that the assessment of the toxicity of a substance by the repeated-dose method is a fundamental test to better assess the safety of this substance.

CONCLUSION

The twelve plants used in the treatment of malaria by traditional healers in the Department of Agboville (South - East of Côte d'Ivoire) are not immediately toxic. As a result, the doses used in this study could be tolerated by the body and would not cause any harm in humans. However, subacute and chronic toxicity studies should be expanded to better evaluate the safety of these plant extracts.

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AUTHOR'S CONTRIBUTIONS

OMR: participated in the botanical survey and in vivo toxicity study

KGR: participated in the drying and preparation of extracts

SGD: took part in the breeding and daily maintenance of rats

KKD: participated in plant harvesting and maintenance of breeding cages

NK: to participate in the clinical observation of animals during and after the administration of drugs.

DAJ: have been involved in drafting the manuscript or revising it critically for important intellectual content

ZGN: have given final approval of the version to be published.

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